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OZONE DISINFECTION OF ESCHERICHIA COLI IN ACTIVATED SLUDGE EFFLUENT

by

GORDON R. FINCH

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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OF DOCTOR OF PHILOSOPHY
IN
ENVIRONMENTAL ENGINEERING

DEPARTMENT OF CIVIL ENGINEERING

EDMONTON, ALBERTA
FALL 1987

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled OZONE DISINFECTION OF ESCHERICHIA COLI IN ACTIVATED SLUDGE EFFLUENT submitted by GORDON R. FINCH in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ENVIRONMENTAL ENGINEERING.

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ABSTRACT

The occurrence of antibiotic-resistant Escherichia coli in raw municipal wastewater, activated sludge effluent, and ozone disinfected activated sludge effluent was investigated using standard antibiotic disc susceptibility tests. Twelve antibiotics representing seven categories of antimicrobials, were used in the investigation based on their clinical use for treating infections caused by members of Enterobacteriaceae. It was found that the activated sludge process selected for multiple-antibiotic-resistant (MAR) E. coli. Ozone disinfection of the effluent using a pilot-scale, semi-batch stirred tank reactor (BSTR) was capable of reducing the fæcal coliform concentration by 99 to 99.9% without selecting for MAR E. coli as has been reported by others for chlorine and ultraviolet radiation. Dose-response experiments involving MAR E. coli and fæcal coliforms found that no differential selection was discernable for ozone doses ranging from 0.4 to 10 mg/L. The dose-response of fæcal coliform bacteria was similar to published data, however the estimates of the empirical model parameters reported by others were statistically different when compared using the 95% joint confidence region of the parameters estimated in this study.

To help understand the causes of the differences in the empirical models, the ozone dose-response of E. coli was investigated using ozone demand-free phosphate buffer and secondary effluent at bench-scale by means of a nalidixic acid-resistant mutant of E. coli (ATCC® 11775). The effect of scale-up and change in the reactor configuration was investigated by scaling-up the bench-scale experiment to a pilot-scale BSTR using the same strain of E. coli. It was found that the utilized ozone dose provided the best correlation with disinfection efficiency in the region from 0 to 99.99% removal and the dose-response was very similar, independent of scale or chemical reactor type. The empirical dose-response model commonly used to size ozone systems for wastewater disinfection was found to have lack-of-fit when applied over the entire dose-response region in both ozone demand-free phosphate buffer and secondary effluent irrespective of scale or ozone reactor type. Consistent disinfection performance was associated with the detection of an ozone residual by the indigo colorimetric method at the end of the mass transfer time period.

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List of Nomenclature

Symbol	Description
ANOVA	analysis of variance
APHA	American Public Health Association
ASCE	American Society of Civil Engineers
ATCC®	American Type Culture Collection
b_i	estimate of the model parameter, β_i
BOD_5	5-day biochemical oxygen demand, mg/L
BSTR	semi-batch stirred tank reactor
c	autocorrelation correction factor for SE($\bar{x}$)
C	concentration of substance, mass/unit volume
C_g	concentration of ozone in the gas phase, mg/L
C_A	applied concentration of ozone, μg/L or mg/L
C_R	residual concentration of ozone, μg/L or mg/L
C_T	transferred concentration of ozone, μg/L or mg/L
C_U	utilized concentration of ozone, μg/L or mg/L
CFU	colony forming units on membrane filter
COD	chemical oxygen demand, mg/L
CV	coefficient of variation, %
DOC	dissolved organic carbon, mg/L
DPD	N,N-diethyl-1,4-phenylene-diamine sulphate
EPA	Environmental Protection Agency
FC	fæcal coliform
ISA	Iso-sensitest agar
k	reaction rate constant
LOF	lack-of-fit

m-FC	fecal coliform agar
m-T7	Tergitol 7 agar
MAR	multiple-antibiotic-resistant
$MAR_{\geq i}$	resistant to ≥ i antibiotics simultaneously
MF	membrane filtration
MPN	most probable number
n	empirical constant for Chick-Watson model
n	sample size used to estimate probability distribution parameters
N	the observed number of CFU/dL after disinfection
N_0	the observed number of CFU/dL before disinfection
NA	nutrient agar
NA_{50}	nutrient agar containing 50 μg/mL of nalidixic acid
NA_{100}	nutrient agar containing 100 μg/mL of nalidixic acid
NCCLS	National Committee for Clinical Laboratory Standards
N/R	not reported
Nal^R	nalidixic acid-resistant strain
Nal^S	nalidixic acid-sensitive strain
OZ	ozone treated secondary effluent
PB	phosphate buffer
PBS	phosphate buffered saline
PE	pure error
PPTG	pour plate in glucose extract agar
PPNA	pour plate in nutrient agar
P_0	proportion of MAR E. coli before ozone disinfection
P_i	proportion of MAR E. coli observed at dose level i
Pr	probability
Q_g	gas flow-rate, L/h (STP)

r	reaction rate, concentration/time
R	raw wastewater
R^2	the proportion of the total variation about the mean explained by the regression
SD	standard deviation
SE	secondary effluent
$SE(b_i)$	standard error of the parameter estimate
$SE(\bar{x})$	standard error of the estimate of the mean
STA	sensitive to all antibioitcs
STP	standard temperature and pressure, 273.15° K and 101.33 kPa
t	contact time, s or min
t_g	elapsed time of ozone gas flow, min
TBA	tryptone bile agar
TKN	total Kjeldahl nitrogen, mg/L
TLPY	tryptose, lactose, proteose peptone, yeast extract agar
TOC	total organic carbon, mg/L
TSB	tryptone soya broth
TSS	total suspended solids, mg/L
UV	ultraviolet radiation
V	volume, L
V_s	volume of stock ozone solution
WPCF	Water Pollution Control Federation
X_i	general independent variable
Y	general random variable
$\bar{x}$	estimate of the mean
β_i	model parameter associated with independent variable i

ρ_1	lag 1 autocorrelation coefficient
σ^2	population variance
Ω_M	oxidation competition value of solute M, mg/L

1. GENERAL INTRODUCTION

1.1 WATER POLLUTION CONTROL

1.1.1 History

Interest in the treatment of municipal wastewater was stimulated in the late nineteenth century for public health and aesthetic reasons. The disposal of raw sewage into convenient lakes or streams resulted in unsightly conditions and a potential risk to public health from users of these surface waters (Metcalf and Eddy, 1979). Kinnicutt et al. (1910) wrote:

> The primary object of sewage purification is the oxidation of organic matter, its conversion into a stable form so that it will not putrefy and create a nuisance. This end can often be attained by methods which do not effect a very notable reduction in the number of bacteria present. In many cases, such methods are well suited to local conditions. Where, for example, the effluent from a purification plant is discharged into a small stream which is not used as a source of water supply, organic stability and removal of suspended matter is all that can properly be demanded. In other instances, however, bacterial purification may be desired. Where an effluent is discharged into the ocean in a strong current of water, suspended solids and organic matter may prove inoffensive; but if there be important shellfish beds in the neighborhood, bacterial purification is an imperative necessity.
>
> The relative importance of freedom from solids, organic stability, and bacterial purity must be determined in each particular case by a study of local factors.

Fair et al. (1968), 58 years later quantified this philosophy into a summary table which classified the degree of wastewater treatment on the basis of the receiving waters' use. Table 1.1 was adapted from this source. Where bacterial purification was considered necessary, chlorination was the process of choice.

1.1.2 Wastewater Treatment Technologies in Canada

Ross et al. (1984) summarized the operation and maintenance costs of municipal wastewater treatment in Canada and defined three categories of treatment:

1. primary treatment;

TABLE 1.1 Indications for degree of wastewater treatment as a function of receiving water use (after Fair et al., 1968).

Water Use	Required Treatment of Water Before Use	Required Treatment of Wastewater Before Discharge	
		Normal	Emergency
Industrial uses, irrigation, avoiding nuisance conditions in surface waters	Sometimes none, chlorination as needed	Sedimentation and chlorination	Heavy chlorination for H_2S removal; addition of nitrate for oxygen supply
Fishing, recreational boating, raising seed oysters, industrial use after treatment	Withdrawal of water not implied	Sedimentation, precipitation or biological treatment depending on the degree of dilution	Aeration, addition of diluent water, heavy chlorination
Bathing, recreation	Withdrawal of water not implied	Sedimentation, precipitation or biologicial treatment depending on dilution, chlorination of effluent	Heavy chlorination
Shellfish culture	Shellfish from restricted areas to be sold only after self-purification in mildly chlorinated water or by transplanting in clean water	Sedimentation, precipitation or biological treatment depending on dilution, chlorination of effluent	Heavy chlorination
Drinking water and domestic use	Depending on raw water quality, pre and post chlorination, coagulation, sedimentation, and filtration	Sedimentation, precipitation or biological treatment depending on dilution, chlorination of effluent	Heavy chlorination, "boil water" orders issued by health authorities

a. screening and primary sedimentation

2. secondary treatment;
   - a. conventional activated sludge
   - b. extended aeration
   - c. contact stabilization
   - d. step aeration
3. lagoons;
   - a. aerated
   - b. facultative

Primary treatment can remove approximately 90% of settlable solids, 50 to 65% of total suspended solids (TSS), and 25 to 50% of 5-day, biochemical oxygen demand (BOD_5) (Pavoni, 1977). Secondary treatment processes are intended to remove 80 to 90% of suspended solids and BOD_5. The water quality parameters defining secondary treatment are (Pavoni, 1977): BOD_5, <30 mg/L; and TSS, <30 mg/L. Lagoon treatment can provide water quality which exceeds or equals either of the two previous categories depending on the design and operation of the lagoon system (Smith and Finch, 1985).

Approximately 6 GL/d of municipal wastewater are treated in Canada. Of this wastewater, 60% is treated by activated sludge, 20% by primary treatment, and 16% by lagoons (Ross et al., 1984). Approximately 90% of the lagoon systems treat wastewater flows of less than 10 ML/d. However, there are more than 800 lagoons operating in Canada compared with 450 activated sludge plants and 74 primary treatment facilities. Therefore, the principal form of treatment with respect to the volume of wastewater is the activated sludge process.

The primary treatment objectives in Canada are removal of biochemical oxygen demand, suspended solids, and phosphorous (Ross et al., 1984). Control of microorganisms in effluents may be required in cases where there is a risk to public health such as discharges into surface waters used for potable water supply or for primary contact recreation (Health and Welfare Canada, 1984).

1.2 PUBLIC HEALTH SIGNIFICANCE OF WASTEWATER EFFLUENTS

1.2.1 Types of Excreted Pathogenic Microorganisms and their Survival

Feachem et al. (1981, 1983) reviewed the health significance of excreta and wastewater management for the World Bank. Their exhaustive review of the literature critically examined the occurrence of some of the most commonly excreted pathogens including the life cycle, modes of transmission, persistence, occurrence in water and soil systems, and survivablity in the most common wastewater treatment systems. Table 1.2 was adapted partly from their reports. Helminth data for Canada were obtained from a National Research Council of Canada report (NRCC, 1980).

It can be seen that for all classes of pathogens, primary treatment was inadequate. Activated sludge was also inadequate for most classes of pathogens. However, helminth ova removal may be significant but no information was found which supports these data under Canadian conditions. Lagoons were the only class of treatment which were able to obtain large reductions in pathogenic organisms. Chlorine disinfection of effluents was considered ineffectual for controlling the pathogenic organisms in virtually all cases. Organisms which were discharged into a fresh water environment were found to persist for periods of several weeks to several months.

Persistence of microorganisms in the aquatic environment has been reported to be principally a function of temperature and time (Feachem et al., 1981; Sattar, 1981). Davenport et al. (1976) reported indicator persistence 2.7 to 5.4 times longer in arctic rivers than previously reported for southern winter conditions. Smith and Gerard (1981) reported negligible coliform die-off when modelling lagoon effluent discharges into an ice-covered river. Dahling and

TABLE 1.2 Some pathogenic organisms found in fæces and their survival in various aquatic environments (after Feachem et al., 1981, 1983; NRCC, 1980).

Organism	Disease	Primary sedimentation (% removal)	Activated sludge (% removal)	Lagoon (% removal)	Effluent chlorination survival	Survival in fresh water
Viruses		0-30	90-99	99.99-100	Yes	Several weeks
Adenoviruses	Various ailments					
Coxsackie viruses	Various ailments					
Echoviruses	Various ailments					
Hepatitis A virus	Infectious hepatitis					
Norwalk agent	Gastroenteritis					
Polioviruses	Poliomyelitis					
Reoviruses	Various conditions					
Rotaviruses	Gastroenteritis					
Bacteria						
Campylobacter fetus	Gastroenteritis	No information	available on	*C. fetus*		
Escherichia coli	Gastroenteritis	50-90	90-99	>99.99	Some survive, may regrow	Several weeks
Salmonella spp.	Salmonellosis	50-90	90-99	99.99-100	No	Several weeks
S. typhi	Typhoid fever					
S. paratyphi	Paratyphoid fever					
Shigella spp.	Shigellosis	50-90	90-99	99.99-100	No	Several weeks
Vibrio cholerae	Cholera	50-90	90-99	100	No	Several weeks
Yersinia enterocolitica	Yersiniosis	No information	available on *Y.*	*enterocolitica*		
Protozoa						
Balantidium coli	Balantidiasis	No information	available on *B.*	*coli*		
Entamoeba histolytica	Amebiasis	10-50	50	100	Yes	<3 weeks
Giardia lamblia	Giardiasis	Believed to be	similar to *Ent.*	*histolytica*		
Helminths		50-70	80-100	100	Yes	Several months
Echinococcus granulosus	Hydatidosis					
Ech. multilocularis	Hydatidosis					
Enterobius vermicularis	Enterobiasis					
Hymenolepis nana	Hymenolepiasis					

Safferman (1979) found enteric viruses had a survival rate of 34% over a 320 km reach of the Tanana River in Alaska. This supported earlier findings by Prier and Riley (1966) who found that Echovirus, Coxackievirus, and Poliovirus were stable in 4° C river and impoundment waters. Katzenelson (1978) stated that viable viruses can be stored indefinitely at deep-freeze temperatures and expected long survival times at 0° to 5° C. Clarke et al. (1962) described data which suggested viruses may remain viable for greater than 200 days at temperatures less than 4° C. They also found that Poliovirus survived three times longer than Escherichia coli and Streptococcus fæcalis. Bitton (1978) reported virus survival of 40 to 90 days at 3° to 5° C under laboratory conditions.

The persistence of microbes in cold water environments, both wastewaters and receiving waters, is of importance in Canada where winter conditions can prevail for up to seven months of the year. Since the activated sludge process treats the majority of wastewater in Canada and does not remove significant numbers of pathogenic organisms, it may be concluded that considerable numbers of potentially pathogenic organisms are being discharged into the environment each day. These organisms have the potential to survive for long periods of time and may be transported to places far removed from their source.

1.2.2 Indicator Bacteria

The use of indicator organisms resulted from the difficulty of isolating and identifying various water-borne pathogens from the extensive flora of the human intestinal tract. Several different criteria have been proposed for choosing the ideal indicator organism (Barrow, 1981; Bonde, 1966; Cabelli, 1977; Dutka, 1973; Feachem et al., 1983; Scarpino, 1971). Dufour (1984) summarized these criteria as:

1. non-pathogenic;
2. present only when pathogens are present;

3. unable to grow in the aquatic environment;
4. resistant to waste treatment processes to a greater degree than pathogens;
5. easy to detect and enumerate;
6. applicable to all types of water;
7. not be subject to antibiosis;
8. absent from sources other than sewage or be exclusively associated with sewage;
9. present in larger numbers than pathogens;
10. dying at the same rate as pathogens or slightly slower than pathogens; and
11. indicator density should correlate with the health hazard from a given type of pollution.

The coliform group of bacteria have been used as the principal indicator of sewage contamination and disinfection efficiency (APHA, 1985). Olivieri (1982) noted the coliform group had some shortcomings which included:

1. die-off rates which were significantly greater than viruses and protozoa;
2. higher sensitivity to treatment processes than pathogens;
3. aftergrowth potential; and
4. the ability to compete successfully in the environment, altering the ratio between coliforms and pathogens unpredictably.

Haas (1986) pointed out that there was little reliable epidemiological data relating the coliform group of bacteria to outbreaks of water-borne disease. Cabelli et al. (1979) and Dufour (1984) provided the first information on the correlation between swimming-associated illness and the concentration of indicator bacteria in marine and fresh water, respectively. Enterococci were the best indicator in marine waters and E. coli was the best in fresh water. Dufour (1984) concluded that the best indicator organisms for all waters were enterococci, and that E. coli would be equally well suited for application in fresh waters. He also concluded that fæcal coliforms were a poor choice of indicator for monitoring recreational water quality. Miescier and Cabelli (1982) studied the survival of six potential indicator bacteria including enterococci and E. coli in muncipal wastewater treatment processes. They found that enterococci were the best survivors among the bacteria studied, followed by E. coli.

Haas et al. (1985) evaluated the use of acid-fast bacteria and yeasts as improved indicators of disinfection performance. They concluded that these organisms had good potential as alternatives to coliform organisms. However, Haas et al. (1985) noted that the data base for the use of acid-fast bacteria and yeasts as indicator organisms was small and does not yet justify a switch from the coliform system.

The coliform indicator system was critically reviewed by Health and Welfare Canada (1984) during their discussion of the need for wastewater disinfection in Canada. They concluded that:

> The application of total and fecal (sic) coliforms as indicators of fecal (sic) contamination of surface waters is subject to misuse and misinterpretation. Coliforms are an ecologically heterogenous (sic) group of microorganisms, only some of which are consistently and exclusively associated with the fecal (sic) wastes of warm-blooded animals. Fecal (sic) coliforms, although a more feces-specific (sic) indicator than total coliforms, are mainly composed of E. coli and Klebsiella spicatum. Klebsiella spp. cannot be consistently recovered from human feces (sic) and have been found on vegetation, in soils, and in industrial effluents. Consequently, sanitary surveys may be necessary in order to identify the source of bacteriological contamination of surface waters.

Another conclusion by the same group was:

> Although there are recognized inadequacies with the use of existing indicator organisms, they have served to reduce the incidence of disease and infection occurring via the potable water route for a number of years. Until other proven methods of detecting pathogens are available, the use of indicator organisms must continue.

These two quotations illustrate the "misinterpretation" and complacency regarding the purpose and use of the indicator system. For instance, E. coli are the most numerous genus in the fæcal coliform group and are normally exclusively of fæcal origin (Feachem et al., 1983; Sonnenwirth, 1980). Enterococci are a subgroup of the Group D streptococci (fæcal streptococci) and are usually excreted by man and other warm-blooded animals (Erickson and Dufour, 1986). Therefore, detection of E. coli or enterococci indicates fæcal contamination of the water. Bacteriological methods are available which permit reliable estimation of the number of indicator bacteria and some pathogenic bacteria in various types of water using membrane filtration (Erickson and Dufour, 1986). Therefore, the first conclusion quoted above is potentially misleading.

Secondly, with respect to the adequacy of the present indicator system for prevention of water-borne disease, the absence of an indicator organism does not indicate the absence of sewage contamination. Standard procedures for the enumeration of coliform bacteria in treated waters have been shown to underestimate the number of surviving coliforms due to sub-lethal injury (McFeters et al., 1986). In addition, Berg and Metcalf (1978) concluded that viruses could be recovered from disinfected waters in the absence of the usual coliform indicators. Similarly for protozoan cysts, the absence of indicator bacteria does not preclude the presence of such cysts (Feachem et al., 1983; WHO, 1984). Therefore, the second conclusion reached by Health and Welfare Canada (1984) may be somewhat misleading if it is interpreted as recommending maintenance of the status quo.

1.2.3 Antibiotic-resistance in Enterobacteriaceae

Fæcal coliform bacteria are members of the family Enterobacteriaceae which includes several genera which are important water-borne pathogenic organisms including Salmonella spp., Shigella spp., and Yersinia spp. Certain strains of E. coli are also pathogenic (Feachem et al., 1983). In the 1950's, outbreaks of antibiotic-resistant shigellosis in Japan led to the discovery of plasmid-mediated, transferable antibiotic-resistance in bacteria (Anderson, 1968). The discovery that these antibiotic-resistant pathogens could pass and receive the plasmids from non-pathogenic, related bacteria such as the indicator bacteria comprising the coliform group, prompted research in the 1970's, notably by Grabow and his coworkers (Grabow et al., 1973; Grabow and Prozesky, 1973; Grabow et al., 1975, 1976) into the environmental occurrence and survival of indicator bacteria which had transferable antibiotic-resistance.

The spread of these organisms in the environment is cause for concern since antibiotic therapy can be adversely affected (Cohen and Tauxe, 1986; Lambert, 1984; Rowe and Threlfall, 1984). For instance, a large outbreak of typhoid fever in Mexico was caused by a multiple-resistant (4 antibiotics) Salmonella strain (Anderson, 1975). Another example was an outbreak of antibiotic-resistant shigellosis in Guatemala (Gangarosa et al., 1972). A major outbreak of milk-borne salmonellosis in the United States involved an organism with plasmid-mediated, multiple-antibiotic-resistance (Ryan et al., 1985; White et al., 1985). More recently, an outbreak of food poisoning in California was caused by a multiple-resistant strain of Salmonella newport in hamburger which originated with slaughtered dairy cows (Spika et al., 1987). The strain was resistant to ampicillin, tetracycline, and chloramphenicol. Animal waste lagoons at farms where animals were fed chloramphenicol were more likely to have chloramphenicol-resistant Salmonella. Holmberg et al. (1984) reviewed the cases of antibiotic-resistant non-typhoid Salmonella which had been reported in the United States from 1971 to 1983. They noted that mortality occurred in 4.2% of the cases caused by antibiotic-resistant Salmonella compared with 0.2 % in cases where the organism was sensitive to antibiotics. Ryder et al. (1980) studied changes in the antibiotic-resistance profile of S. typhimurium from 1967 to 1975 in the United States. There was a significant increase in the frequency of Salmonella strains resistant to streptomycin, sulphonamides, ampicillin, kanamycin, and chloramphenicol. There was also a significant increase in Salmonella strains with simultaneous resistance to six or more antibiotics.

The potential for domestic animals to contract salmonellosis from wastewater discharges was demonstrated by Reilly et al. (1981) who examined 26 pollution-associated incidents of salmonellosis in Scotland between 1973 and 1979. Humans were affected in 3 of the outbreaks and effluents from municipal and industrial treatment works accounted for 46% of the incidents while septic tank discharges accounted for 31%. Cattle infection with Salmonella was observed in 23 of the outbreaks and was found to be associated with sewage effluents. Humans were affected in the other 3 outbreaks. S. typhimurium was the predominant strain implicated in the infections. Antibiotic-resistance was not reported.

Williams-Smith (1970) concluded that transmission of antibiotic-resistant enteric bacteria by means of wastewater was the main source in the aquatic environment. He noted that urban wastewater contributed more antibiotic-resistant E. coli to receiving waters compared with rural areas. Bell et al. (1983) reported similar observations. In a review paper, Grabow et al. (1974) suggested that the discharge of large numbers of non-pathogenic indicator bacteria with transferable antibiotic-resistance into the environment may lead to broad environmental problems which may pose a threat to public health. Therefore, adequate treatment of excreta is an important element in the control of water-borne bacteria with transferable resistance factors since wastewater is responsible for disseminating large numbers of these organisms into the aquatic environment (Bell et al., 1980, 1981; Grabow and Prozesky, 1973; Grabow et al., 1976; Murray et al., 1984). Grabow et al. (1974) and Bell et al. (1981) suggested that biological water quality criterion for wastewater treatment works be reviewed in relation to the occurrence of bacteria with transferable antibiotic-resistance.

A report by Health and Welfare Canada (1984) on the status of wastewater disinfection in Canada recognized that wastewater effluents were a significant source of pathogens in the receiving environment and may constitute a health hazard in the subsequent use of receiving waters for recreation, water supply and agriculture. Notably, further study of antibiotic-resistant indicator bacteria in wastewater was recommended. Also, the effects of wastewater disinfection on the survival of antibiotic-resistant indicator bacteria was felt to be poorly understood and required further study.

1.2.4 Public Health Risks Associated with Wastewater Discharges

Warner (1984) defined risk assessment as the study of decisions subject to uncertain consequences. The topic was sub-divided into risk-estimation and risk-evaluation. Risk-estimation comprised:

1. the identification of the outcomes;
2. the estimation of the magnitude of the associated consequences of these outcomes; and
3. the estimation of the probabilities of these outcomes.

Risk evaluation was the process of assigning a value to the possible outcome of the decision. For example, incidences of swimming-associated gastroenteritis resulting from swimming in sewage-contaminated surface water could be evaluated on the basis of the cost to society for treating the illnesses and loss of productivity versus the cost of reducing the incidences of illness through improvement of waste treatment.

An early discussion of the risk of illness associated with wastewater discharges was a report by the ASCE Public Health Activities Committee (1963). They concluded that the setting of rational surface water quality standards was impossible until proper epidemiological correlations had been made, particularly since a great deal of capital investment and operating costs were associated with the disinfection of wastewater effluents. They also concluded that the existing coliform standard of 1000/mL had not been directly associated with the risk of contracting an illness.

Cabelli et al. (1979, 1982) established epidemiological evidence relating outbreaks of swimming-associated gastroenteritis to the mean density of enterococci in marine waters. A 1% incidence rate was associated with less than 10 enterococci per dL. Dufour (1984) reported good correlation between the density of E. coli and the risk of illness amongst swimmers in freshwaters finding a 1% incidence rate of illness associated with E. coli and enterococci densities of less than 300 and 70/dL, respectively. Haas (1986) discussed the risk of contracting illness from wastewater discharges into surface waters which were used for recreation and potable water supplies. He reviewed current indicator systems and their correlation with disease and demonstrated how to perform a quantitative risk estimation using each system. Haas (1983) used this methodology to assess the risk of contracting viral illness resulting from undisinfected wastewater discharges into surface waters used for primary contact recreation. He concluded that stopping wastewater disinfection in the State of Illinois would result in a significant increase in the number of swimming-associated cases of gastroenteritis.

Logsdon and Hoff (1986) noted that there were two principal barriers for the prevention of water-borne disease: a source of raw water with acceptable water quality; and a water treatment system capable of meeting drinking water standards consistently. Challenges from microbial contamination of the water supply may break through an inadequate or indifferently operated water treatment plant (Lippy, 1986). Craun (1986a) documented the number of cases of water-borne illness in the United States from 1920-80. The most common cause of illness was inadequate or interrupted treatment of the water supply. Aetiological agents were identified in 56% of the outbreaks. The water-borne illnesses most frequently observed during this 60 year period were acute gastroenteritis of unknown aetiology, typhoid fever, shigellosis, hepatitis A, and giardiasis. For the period 1981-83, 60% of the outbreaks of water-borne disease had an aetiological agent identified (Craun, 1986b). Of these, Giardia lamblia was the most common agent. Acute gastroenteritis of unknown aetiology remained a major cause of illness resulting from water-borne agents.

Finch et al. (1985) in a review of water filtration technologies concluded that rapid sand filtration of surface water supplies without adequate pretreatment, such as optimized coagulation/flocculation, would not remove Giardia sized particles. Furthermore, it is believed that Giardia cysts are resistant to chlorine residuals normally used in water treatment plants, particularly at low temperatures (Hoff, 1986; WHO, 1984). Consequently, the disinfection barrier in water treatment plants may be breached if surface water supplies have been contaminated by undisinfected or inadequately disinfected wastewater discharges.

In Canada, water quality guidelines for primary contact recreation in surface waters are generally less than 1000 total coliforms per dL or less than 200 fæcal coliforms per dL (Health and Welfare Canada, 1984). Currently, there are no limits on indicator bacteria in raw water supplies if complete water treatment treatment consisting of coagulation, sedimentation, filtration, and chlorination is performed. In regions where there are shellfish industries, the guidelines recommend less than 14 fæcal coliforms per dL (Health and Welfare Canada, 1984).

1.3 CONTROL OF MICROORGANISMS IN WASTEWATER EFFLUENTS

Disinfection of wastewater effluents has been extensively studied in the United States since the early 1970's in response to the inclusion of a universal fæcal coliform standard for wastewater discharges in the Clean Water Act of 1972 (Haas, 1986). The potentially adverse effects caused by the extensive use of chlorine as a wastewater disinfectant was recognized by the Canadian and United States governments (Environment Canada, 1978; United States EPA, 1976) and led to the discontinuation of the universal disinfection requirement in the United States in 1976. The search for effective alternatives to chlorine resulted in the evaluation of other halogens, ultraviolet radiation, and ozone as technically feasible processes (WPCF, 1984). Ultraviolet radiation and ozone have been considered the most feasible alternatives to chlorination. Both have demonstrated effectiveness for reduction of pathogenic organisms in the water and wastewater environment. However, practical engineering experience with both systems is limited, leading to some design and performance problems (WPCF, 1986). These problems are gradually becoming better understood and new systems have a better chance of performing to expectations.

Extensive reviews of ozone disinfection of water and wastewater have been published by Kinman (1975), Rice et al. (1982), and Venosa (1972). The conclusions reached in these studies were that ozone was a very effective disinfectant but its use was not economically competitive with other methods. Also, concerns about efficient reactor design, ozone analysis, and basic disinfection dose-response and kinetic relationships were expressed. More research was recommended in fundamental topics such as reactor design, toxicity to aquatic organisms, disinfection mechanisms, and reaction products.

In the 12 to 15 years since these reports were completed, several of these issues have been addressed. WPCF (1986) summarized research results regarding the toxicity of ozone residuals to various aquatic organisms. It was concluded that the toxic response was no worse than that for chlorine residuals and that at the concentrations normally found in wastewater effluents, where residuals are short-lived, the likelihood of adverse environmental effects was small.

Attempts have been made to describe the changes in the organic constituents of secondary effluent during ozone disinfection (Hoigné and Bader, 1978; Legube et al., 1987). The variety of organic compounds in biologically treated effluents causes difficulties in determining the changes which occur and the implications of the changes. More work is needed in this area.

As will be seen in the next section, analytical methods for the determination of ozone in the gas and aqueous phase have improved during the past few years, yet they are only on the verge of being accepted as standard methods. Similarly, controversy still remains regarding the choice of ozone equipment and the design of ozone contactors.

1.4 DISINFECTION WITH OZONE

1.4.1 Mechanism for Bacterial Disinfection Using Ozone

The evidence from the literature is contradictory regarding the mode of action of ozone. Some studies indicated that ozone alters proteins and the unsaturated bonds of fatty acids in the cell membrane, adversely affecting the permeability (Pryor et al., 1983; Scott and Lesher, 1963; Smith, 1967). However, others have provided evidence for ozone affecting DNA in the cell, causing cell inactivation (Hamelin and Chung, 1974, 1976; Ishizaki et al., 1987; Ohlrogge and Kernan, 1983). Evidently the mode of action of ozone is complex, and further research is necessary before the mode of action can be clearly defined.

1.4.2 Ozone Chemistry in Aqueous Solution

Two main reaction schemes have been proposed for ozone in water (Hoigné and Bader, 1975). The first mechanism is the direct oxidation of compounds by the ozone molecule. These reactions are quite selective and can take several minutes. Therefore, in the absence of ozone decomposition the ozone concentration should remain relatively constant over short time intervals. The second mechanism is through the oxidation of chemical compounds by ozone decomposition products, principally, the hydroxyl radical. This radical is highly reactive and has a life span of only a few microseconds in water. Ozone decomposition is influenced by high pH and temperature. Increases in these factors rapidly decrease ozone in solution. However, the presence of radical scavengers such as carbonate and bicarbonate ions (alkalinity), can reduce the rate of decomposition of ozone by quenching the chain reaction initiated by the hydroxyl radicals (Doré et al., 1987; Hoigné, 1982).

Hoigné (1982) defined the oxidation competition value, Ω_M, as the amount of decomposed ozone required to reduce the concentration of solute M to 37% of its initial value. Ω_M is influenced by the type of water (Hoigné and Bader, 1979b). In particular, Ω_M increases linearly with the concentration of hydroxyl-radical-consuming compounds (Hoigné, 1982). Hoigné and Bader (1979b) reported that Ω_M increased as a function of the dissolved organic carbon (DOC) concentration and found that Ω_M increased 0.4 mg/L per mg/L of DOC present in Swiss surface water and wastewater. The significance of this observation is that the degree of conversion of a solute decreases with increasing competition for ozone as measured by the DOC content.

Knowledge of Ω_M for one solute permits calculation of the Ω_M for another solute if the relative reaction rate constants are known (Hoigné and Bader, 1979a, 1979b). Conversely, if the Ω_M is known for different solutes, the relative reaction rate constant can be calculated. This fact permits determination of an unknown reaction rate constant from information regarding the relative Ω_M values and the reaction rate constant of one solute (Hoigné, 1982). This methodology was used by Hoigné and Bader (1983a, 1983b) and Hoigné et al. (1985) to compile reaction rate constants for reactions between ozone and a variety of organic and inorganic chemical compounds of interest in water and wastewater treatment. Once the reaction order and reaction rate constant are known, ozone chemical reactors can be designed using standard complete mix or plug flow reactor theory to achieve the desired conversion of a target solute.

Hoigné and Bader (1975, 1978) stated that disinfection was the result of direct ozone oxidation and not the radical mechanism as suggested by Dahi

(1976). Bancroft et al. (1984) postulated that the disinfection reaction was hydroxyl radical mediated but their evidence was not sufficient to verify their theory. Ross et al. (1976) and Venosa (1972) reported that pH had little or no effect on ozone disinfection of water suggesting that the direct ozone reaction predominates. Further research into the mode of action of ozone on bacterial cells is necessary to discover if there is a disinfection rate-limiting chemical reaction which controls disinfection. Once this hypothetical reaction has been found, the reaction rate constant can be evaluated and disinfection process design can be accomplished using standard chemical engineering reactor design procedures.

1.4.3 Ozone Disinfection Kinetics

Modelling chemical disinfection of water originated with studies by Chick (1908) and Watson (1908). Chick (1908) postulated that the disinfection reaction was similar to a bimolecular chemical reaction. The reaction rate was determined by the relative concentrations of microorganisms and disinfectant. She postulated that if the disinfectant concentration was constant, the disinfection reaction would be first-order with respect to surviving bacteria. The "Chick-Watson" model has been expressed as (Haas and Karra, 1984):

$$\log \frac{N}{N_0} = -k\ C^n\ t \qquad [1.1]$$

where N is the concentration of surviving bacteria at time t, N_0 is the initial concentration of bacteria, k is the reaction rate constant, C is the residual concentration of disinfectant, and n is the dilution factor.

Prokop and Humphrey (1970) summarized and discussed the various mechanistic, stochcastic, and empirical models which had been formulated to describe disinfection kinetics and deviations from the Chick-Watson model. However, Haas and Karra (1984) concluded that the Chick-Watson model provided an adequate fit for chlorine disinfection kinetic data collected from demand-free systems.

Katzenelson et al. (1974) demonstrated that ozone disinfection kinetics were different from those predicted by the Chick-Watson model. They found that ozone disinfection had two stages; an initial rapid stage followed by a much slower second stage. A study by Popp (1954) of chlorine disinfection kinetics reported a kinetic relationship similar to that of Katzenelson et al. (1974) except the breakpoint was not as pronounced. Hom (1972) and others (Bancroft et al., 1984; Prokop and Humphrey, 1970; Silverman and Sinskey, 1977) have attributed this phenomenon to populations of mixed organisms with differential resistances to the disinfectant. Chick (1908) suggested that age differences within a bacterial population may account for differential resistances to chemical disinfectants. Leyval et al. (1984) and Seyfried and Fraser (1980) reported chlorine-resistant strains of Pseudomonas aeruginosa in swimming pool waters. They found that isolates of the same strain could be chlorine-resistant. The only difference between strains was an exocellular slime layer on the chlorine-resistant one. Scott and Lesher (1963) found that E. coli in the logarithmic-growth phase were more sensitive to ozone than the same strain in the stationary-growth phase. Others have postulated that microorganism clumping was the cause of the concave-upwards plots of disinfection kinetic data (Dahi, 1976; Katzenelson et al., 1974).

Several investigators have reported ozone disinfection to be relatively independent of contact time after the initial inactivation stage was complete, usually after 30 to 60 s in clean water (Engelbrecht et al., 1979; Katzenelson et al., 1974; Smith, 1967; Sproul et al., 1979). The EPA Wastewater Disinfection Design Manual (United States EPA, 1986) recommended contact times from 6 to 10 min for ozone disinfection systems on the basis of hydraulic considerations rather than process kinetics. This has led to process design based on empirical dose-response models since the concentration of ozone was more important in effecting bacterial reductions than contact time.

1.4.4 Dose-response Concepts in Environmental Toxicology

Brown (1978) and Connell and Miller (1984) summarized the various dose-response models which have been used in environmental toxicology. A common toxic response relevent to disinfection is the "all or none" or quantal response. Stochastic probit and logit models have been used to describe quantal dose-response data collected from bioassays (Finney, 1971). These models assume the responses are lognormally distributed and are random, independent samples from a genetically consistent population of organisms.

Krewski and Van Ryzin (1981) reviewed the commonly used quantal response models for their ability to extrapolate the response of an organism to low doses of a toxicant. They concluded that although the basic probit and logit models and more sophisticated multi-hit models estimated the same response for mid-range dose levels, extrapolation to low doses produced vastly different estimates of response depending on the model used.

However, the underlying assumptions of the response distribution and the test organisms must be carefully considered. For example, each bacterium should be the same strain and age. Also, each bacterium should be individually distributed in solution and exposed to equal concentrations of disinfectant. Finally, the proportion responding to a specified dose of disinfectant should be independent of the initial concentration of viable organisms.

Finney (1971) used a probit model to describe heat sterilization of bacteria using the data of Wadley (1949). A solution of bacteria was held at 107° C and was sampled for surviving bacteria at prescribed time intervals from 0 to 50 min. The sample sizes were such that the initial number of bacteria was 233 and the final number after 50 min of heating at 107° C was 3 bacteria, or 1.3% survival. Using the probit model, Finney then estimated the effective dose for reducing the bacteria by 50%.

The difficulty in applying this method to water disinfection is that 1.3% survival of bacteria represents less than 2 log units of reduction. In practical water and wastewater problems, frequently 3 to 4 log unit reductions are necessary. Hence the response level of interest is 99.9 to 99.99%, not 50%. These responses are in the region where quantal response models have not been examined in normal bioassays because of practical limitations on the number of subjects. Therefore, the study of toxic responses in bacteria may provide the means for discriminating between the various quantal response models since large numbers of subjects are available for testing.

Scott and Lesher (1963) suggested there was a stoichiometric relationship between E. coli and ozone. The implication of this observation is that the initial concentration of bacteria will determine the proportion responding to a given concentration of ozone. Whereas a toxic response would be dependent on the distribution of resistances to the toxin amongst the bacterial population independently of the number of subjects exposed to the toxin. Therefore, it is possible that dose-response models from the toxicology literature will have little application in chemical disinfection of water and wastewater.

1.4.5 Process Design for Ozone Disinfection of Wastewater

Process design remains an empirical problem, because the mode of action, chemical reactions and chemical kinetics of ozone and bacteria are not fully understood. Generally, the recommended design approach for ozone oxidation or disinfection processes consists of costly pilot-plant studies (Rice et al., 1982; United States EPA, 1986). Happenstance data† collected from these pilot-plant studies is typically analysed using multiple-regression analysis. The general form of the empirical model is (Caverson et al., 1986):

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† Data collected at random from a process without due regard for statistical experimental design is known as happenstance data.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 \qquad [1.2]$$

where Y is bacterial survival and $X_{1,2}$ are dose and wastewater strength metameters,[‡] respectively. Smith and Given (1979) were among the first investigators to use this model for ozone disinfection studies.

Investigators who have used multiple-regression analysis rarely perform the diagnostic tests for model adequacy and checking the underlying assumptions of the method of least-squares. For example, Meckes _et al_. (1983) published the results from a study of ozone disinfection on six different wastewater effluents. Figures presented in the publication plotted the mean response values for total coliforms and the calculated regression lines. It was claimed that the model was a good predictor of disinfection performance (WPCF, 1984). However, no evidence was given for this statement. Examination of the raw disinfection data from the study of Meckes _et al_. (1983) indicated that the variance of the disinfection response to ozone was very large. This may have been the result of variations in wastewater quality since all of the plants studied were relatively small. Analysis of the disinfection data without consideration for wastewater strength (COD), revealed that three of the wastewaters did not have significant dose-response regressions ($Pr \leq 0.01$). The other three wastewaters produced adequate dose-response models. Also, the parameter estimates were highly correlated suggesting that the experimental design may be very important in determining precise estimates of the model parameters. This example illustrates the care that is required in regression analysis if a meaningful conclusion is to be drawn from the data.

As shown and discussed by Caverson _et al_. (1986), Rakness _et al_. (1986a) and the United States EPA (1986), the precision of the model parameter estimates can significantly affect the estimation of the ozone dose required to achieve a specified reduction in bacteria.

[‡] Finney (1971) defined metameter as the transformed scale of dose or response in bioassay.

The precision of the parameter estimates can be greatly improved if adequate experimental design is used (Draper and Smith, 1981). The hazards of using happenstance data and regression analysis to determine cause-effect relationships has been discussed by Box et al. (1978). This is particularly important when investigating the response of biological populations to a stimulus. In these cases, the natural response distribution has a relatively large variance compared with data gathered from chemical processes (Finney, 1978). This problem is compounded by fluctuations in wastewater quality which influences the ozone demand and subsequently, disinfection performance.

There is a need for an experimental protocol which provides the maximum amount of process design information using the least amount of experimental effort.

1.4.6 Components of an Ozone Disinfection System

The design of a wastewater disinfection facility is comprised of two fundamental functions: facilities design and process design. Facilities design consists of five major components (Rakness et al., 1987; Robson, 1982):

1. feed-gas preparation;
2. ozone generation;
3. ozone contacting device;
4. off-gas treatment; and
5. process control.

Years of experience with ozone water treatment in Europe has provided a wealth of practical facility design experience, which has been documented most recently by Rice and Netzer (1982). The International Ozone Association (1986, 1987a), through design workshops, is attempting to disseminate this accumulated practical experience to designers of ozonation systems in an effort to produce more reliable and economical ozone systems. Some of the pertinent issues associated with each system component will be discussed in the following sub-sections.

1.4.6.1 Ozone generation

Ozone is an unstable chemical compound and is usually produced in the presence of oxygen or oxygen-containing compounds at the point-of-use. A variety of methods have been used to generate ozone including (Horváth et al., 1985; Rice and Netzer, 1982):

1. electrical discharge;
2. photochemical use of ultraviolet radiation; and
3. electolytic reactions.

The most common commercial means of generating ozone is by corona discharge since it is currently the most economical method for producing large amounts of ozone.

The yield of ozone from a corona discharge generator is dependent upon a variety of factors. The most important of these are (Carlins and Clark, 1982; Horváth et al., 1985):

1. the feed-gas oxygen concentration;
2. power density at the dielectric (generally a function of alternating current frequency, driving voltage and dielectric thickness);
3. temperature at the dielectric;
4. dew point of the feed gas;
5. presence of gas diluents such as nitrogen or hydrogen;
6. the feed-gas flow-rate through the generator; and
7. the pressure of the gas in the generator.

The various equipment manufacturers such as BBC-Brown Boveri, Trailigaz or Degrémont have developed optimum proprietary designs. If high quality feed-gas is used, good performance can be obtained from commercial corona discharge generators.

Measuring ozone generator performance depends on the accurate and precise measurement of ozone in the gas phase. Typically, iodometry has been used to determine the mass of ozone in an accurately measured volume of gas. However, difficulties with the stoichiometry of the iodide reactions for the various iodometric methods have resulted in methods which may give inaccurate results (Grunwell et al., 1983). The United States EPA (1978) adopted ultraviolet spectrophotometry as the absolute standard for atmospheric ozone measurement replacing the neutral buffered iodometric method (Rehme et al., 1980).

The International Ozone Association (1987b) has recommended two standard methods for the determination of ozone in the gas phase. The first is ultraviolet spectrophotometry based on a molar extinction coefficient of $2973 \frac{L}{cm \cdot mol}$ at 253.7 nm and standard temperature and pressure (STP; 273.15° K and 101.33 kPa). The second recommended standard method is the neutral buffered iodometric method.

It remains to be decided which methods will be adopted as standard methods for ozone determination in the gaseous phase. In the meantime, it is important to specify the procedure used.

1.4.6.2 Feed-gas

Feed-gas conditioning is an important equipment consideration in an ozone system. Water vapour is a very important factor in feed-gas quality since minute quantities of water vapour can adversely affect ozone yield and the longevity of the dielectric. A minimum dew point of -60° C is required to ensure good ozone yields with minimum dielectric maintenance and longevity. Other characteristics of the feed-gas are also important for the most efficient operation of the ozone generator. Particles greater than 5 μm can cause coating of the dielectric leading to frequent cleaning or premature dielectric failure. Hydrocarbons can create a serious risk of explosion and concentrations should not exceed 25% of the lower explosion limit. Nitrogen significantly decreases the yield of ozone. This fact needs to be considered when recycling process off-gas to the feed-gas preparation system since nitrogen enrichment of the off-gas occurs. The choice of components for the feed-gas preparation system was described in the United States EPA (1986) design manual. However, as discussed at the International Ozone Association (1986, 1987a) design workshops, there is much controversy over the choice of specific equipment items such as drying systems and compressors. The most experienced companies producing ozone generation equipment should be consulted for proven combinations of equipment since much of the information is proprietary.

Another important decision involving feed-gas is the type of gas to use. The choice between oxygen or air is based on site-specific factors, which in turn, affect the economics.

1.4.6.3 Ozone contacting devices

Ozone contacting clearly falls in the realm of gas-liquid reactions which is well documented in the chemical engineering literature (Astarita, 1967; Danckwerts, 1970; Oldshue, 1983; Rase, 1977). Masschelein (1982) produced a comprehensive comparative review of ozone reactors. He concluded that,

> In selecting ozone-water contacting for any particular case, the following fundamental and engineering criteria must be taken into consideration on a comparative basis:
> 1. average vent losses at a given residual ozone concentration in water equivalent to the practical dissolution yield attainable;
> 2. overall costs of operating the systems reported on a reliable basis of ozone consumption;
> 3. flexibility of air-liquid contacting ratio;
> 4. combined action of instant bubble contact and prolonged residual ozone;
> 5. the possibility of combination or repeated or multiple contacting units;
> 6. approach of bubble size distribution to the theoretical effectiveness

of the contacting process;

7. simplicity in prolonged operation vis-a-vis (sic) being complicated by moving parts or clogging of static elements;
8. operation in pressurized or vacuum systems related to the possible escape of off-gases;
9. necessity of protecting contacting basins against corrosion or alterations; and
10. the possibility of up-rating flow conditions in the contact structures.

Table 1.3 was derived from Masschelein's (1982) review and summarizes the major advantages and disadvantages of a variety of ozone contacting systems.

Scaccia and Rosen (1978) concluded that most of the commonly used reactor configurations could be optimally designed to provide equally good mass transfer, ozone utilization, and disinfection and oxidation efficiencies. They also concluded that the transferred ozone dose was the only important parameter in determining disinfection performance.

The United States EPA embarked on a program of contactor evaluation in the 1970's. The results of this program have been reported by Venosa and his colleagues in a variety of publications (Venosa et al., 1979a, 1979b, 1980). They concluded that multiple-injection, bubble diffuser sytems operated with gas flow counter-current to liquid flow produced the best mass transfer. Fundamental chemical engineering concepts indicate that multiple-stage, complete mix contactors approach ideal plug flow conditions (Grady and Lim, 1980). Consequently, the results of the United States EPA program suggest that plug flow systems may be the best for disinfection performance.

Chemical reaction kinetics in batch or semi-batch complete mix reactors are identical to continuous, ideal plug flow regimes (Grady and Lim, 1980). Gurol (1985) advocated the use of semi-batch stirred tank reactors for fundamental process work since the kinetic relationships could be determined and applied to process problems using established chemical engineering concepts.

TABLE 1.3 Summary of various ozone contactors and their relative merits (after Masschelein, 1982).

Contacting Device	Major Advantage	Major Disadvantage	Energy needs, W·h/g O_3
Dispersion with porous elements	Static	Clogging and channelling of gas flow	2-3
Repeated static injection	Static	Critically flow dependent	2-3
Static mixing	Static	Ozone losses	4-5
Sonic mixing	Static	Cost	Unknown
Total injectors	High contact rate	High ozone losses	15-20
Pipe injection	Low cost	Bumping	0-5
Partial injection	High turbulence and dissolution	Partial over and under ozonation	4-45
"Slow downflow" injection	No moving parts	Channelling of gas flow	4-5
Spray towers	Low investments	High ozone losses	Unknown
Packed or plate column	High yields	Clogging and pressure dependence	15-40
Gas impeller	Reliablity	Ozone losses	2-3
Surface turbine	Accessibility	Ozone losses	7-10
Vortex system	Simplicity	Instability and only experimental	4-6
Recirculating turbine	High bubble contacting	Moving turbines	5-7

From the literature, it is apparent that there is not a universal "off-the-shelf" ozone contacting system which can be recommended. Masschelein (1982) succinctly summarized the major advantages and disadvantages of the systems in use. However, the actual design of the contactor remains the responsibility of the designer. Experience in the design and performance of ozone contacting systems will determine if the contacting system is successful or not.

1.4.6.4 Off-gas treatment

Ozone which is not transferred into solution remains in the carrier gas and is vented from the contactor. This ozone mixture can be toxic to plants and animals even at very low concentrations. Therefore, exhaust gases from an ozone contactor need to be treated.

Masschelein (1982) provided a comprehensive review of off-gas treatment procedures. Table 1.4 summarizes the major advantages and disadvantages of the various systems for off-gas treatment and the relative operating costs of each one.

1.4.6:5 Process control

Process control in water treatment involves the maintenance of an ozone residual for a given contact time. The French standard for ozone disinfection of potable water is 0.4 mg/L for 4 min (Légeron, 1984). This criterion was derived from experimental work by Coin et al. (1964, 1967) investigating the inactivation of polioviruses in water by means of ozone.

The majority of methods used to determine ozone residual have relied upon a version of iodometry, using colorimetric or titrimetric procedures. The most commonly used procedures have been iodide/triiodide colorimetry (Schecter, 1973), the N,N-diethyl-1,4-phenylene-diamine sulphate (DPD) method (Palin, 1974) which can be performed either titrimetrically or colorimetrically, and the potassium iodide titrimetric method (APHA, 1985; Birdsall et al.,

TABLE 1.4 Summary of various ozone off-gas treatments and their relative merits (after Masschelein, 1982).

System	Major Advantages	Major Disadvantages	Energy needs, W·h/m³ (STP)
Preozonation	Ozone is used	Partial destruction only	80-150
Recycling	No off-gas to treat	Corrosion risk	80-100
Dilution	Easy operation	Noise problem	8-10
Scrubbing	Safe equipment	Partial reaction only	25-60
Heating	Easy monitoring, good yield	Hot off-gases, difficult to automate	85-170
Combustion	Total destruction	Large equipment	≃150
Activated carbon	Static operation	Explosion risk	10-15
Catalysts	Small equipment	Catalyst poisoning	5
Silica gel	Partial yield	Irreversibility on regeneration	2
Zeolites	Long cycle periods	Experimental	1-2

1952). A major disadvantage in using these methods for ozone residual determination in water is that any oxidant which oxidizes iodide will interfere. The indigo method (Bader and Hoigné, 1981, 1982) has been recommended as a standard method for ozone residual by Gordon and his coworkers (Gordon and Grunwell, 1984; Grunwell et al., 1983). They found that none of the iodometric procedures gave reliable ozone measurements in aqueous solutions under the conditions tested.

Several electrochemical methods have been evaluated for continuous analysis of ozone residuals (Grunwell et al., 1983; Johnson et al., 1986; Stanley and Johnson, 1982). Some investigators reported that membrane electrodes gave unreliable results and required frequent calibration (Grunwell et al., 1983). Others found good reproducibility and selectivity when using membrane electrodes in water and wastewater when compared with the indigo and ultraviolet absorbance methods (Johnson et al., 1986). Other electrochemical devices such as bare amperometric electrode systems have been reported to produce reliable results in drinking water applications (Masschelein, 1987; Paré, 1982).

Process control in wastewater has not been based on maintaining an ozone residual due to the more rapid decay of ozone in wastewater compared with water and because reliable continuous ozone residual measurement devices were not available for wastewater. Venosa and his coworkers pioneered work in disinfection process control through monitoring of off-gas ozone concentration (Venosa and Meckes, 1983; Venosa et al., 1985). Rakness et al. (1986b) reported successful process control using the off-gas technique.

Automated process control requires the capability to continuously monitor the ozone concentration in either the gas phase or aqueous solution. Of the methods discussed for determining ozone in the gas-phase and aqueous phase, ultraviolet spectrophotometry and eletrochemistry provide the best potential, respectively. Robson (1982) discussed some basic process control systems which can be used for ozone disinfection processes. With the advent of powerful microcomputers and sophisticated process control software, there is good potential for precise process control and concomitant savings in operating expenses.

1.5 SCOPE OF RESEARCH PROGRAM

The goals of this investigation were:

1. to ascertain the effect of ozone on multiple-antibiotic-resistant (MAR) E. coli; and
2. to evaluate the process design procedures for ozone disinfection of activated sludge effluents.

Specifically, the following objectives have been defined as relevant to achieving the above goals:

1. determine if the activated sludge process promotes an increase in the proportion of MAR E. coli in the final effluent relative to antibiotic-sensitive strains;
2. determine if MAR E. coli strains are differentially selected compared with antibiotic-sensitive strains during ozone disinfection of activated sludge effluent;
3. determine the reference dose-response relationship of a marker strain of E. coli in ozone demand-free water by means of bench-scale tests;
4. determine the dose-response relationship of a marker strain of E. coli in a typical activated sludge effluent at bench-scale;
5. determine the dose-response of E. coli in the same wastewater at pilot-scale; and
6. evaluate current process design practice for ozone disinfection of wastewater on the basis of the findings.

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2. THE OCCURRENCE OF MAR E. COLI IN AN ACTIVATED SLUDGE PLANT

2.1 INTRODUCTION

Attention has been drawn to the problem of antibiotic-resistant coliform bacteria in water and wastewater (Armstrong et al., 1981; Bell et al., 1983). Antibiotic-resistance stems from either spontaneous mutation or transfer of genetic material by conjugation, transduction, or transformation (Grabow et al., 1974; Joklik et al., 1980). The former type of resistance seldom has a high frequency of occurrence when two or more classes of drugs are used (Day, 1982; Joklik et al., 1980). However, the display of simultaneous resistance to several antibiotics is typical of extrachromosomal segments of DNA known as resistance plasmids or R-factors (Day, 1982).

Transferable antibiotic-resistance in pathogenic bacteria was first investigated in the late 1950's during an outbreak of shigellosis in Japan as was noted by Anderson (1968) in his review paper on transferable antibiotic-resistance in Enterobacteriaceae. It has been documented that resistance plasmids can be transmitted amongst related genera of bacteria by conjugation (Grabow et al., 1974). However, it is known that only plasmids with transfer factors can be transferred by conjugation (Hardy, 1981).

Early research into the prevalence of antibiotic-resistant bacteria in surface waters concluded that municipal sewage disposal was a significant source of antibiotic-resistant organisms (Williams-Smith, 1970). Alcaide and Garay (1984) examined wastewater from a wastewater treatment plant and a polluted surface water. They found that of the 865 Salmonella spp. isolates tested, 12.7% were resistant to one or more of the 7 antibiotics tested and of these, 30% transferred resistance to an Escherichia coli recipient. They also reported a high

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transfer frequency amongst the strains isolated from sewage. Grabow and Prozesky (1973) reported 26% of coliform isolates from hospital sewage had transferable resistance to one or more of the 13 antibiotics tested. This was in sharp contrast to city sewage which contained 4% with transferable antibiotic-resistance. Sturtevant and Feary (1969) reported that 1% of lactose-fermenting bacteria isolated from raw and treated sewage were multiple-antibiotic-resistant (MAR). They found that 50% of these organisms could transfer all or part of their antibiotic-resistance. In another study, Sturtevant et al. (1971) found 3% of total and fæcal coliforms in raw sewage had resistance to two or more antibiotics. Of these, 48% could transfer all or part of their antibiotic-resistance. Meckes (1982) reported that 82% of 300 total coliforms isolated from activated sludge effluent were resistant to two or more antibiotics. Bell et al. (1981) collected a single grab sample from a variety of wastewater treatment facilities in western Canada and determined the proportion of total and fæcal coliforms with transferable antibiotic-resistance in the influents and the effluents. The influent proportion ranged from 5.33 to 10.25% for total coliforms and from 9.03 to 17% for fæcal coliforms. The effluent proportion ranged from 3.16 to 15.69% for total coliforms and from 6 to 13.22% for fæcal coliforms. Walter and Vennes (1985) reported that 5% of the total coliform population in raw wastewater were MAR in June and but decreased to 0.35% in January, suggesting a marked seasonal trend.

Several investigators reported that wastewater treatment had a positive selective pressure on sewage bacteria with antibiotic-resistance (Bell et al., 1981, 1983; Grabow et al., 1973; Grabow and Prozesky, 1973; Kish and Lampky, 1983; Walter and Vennes, 1985). However, contradictory evidence has also been reported which indicated that there was no change or a decrease in the proportion of antibiotic-resistant bacteria in wastewater treatment (Bell, 1978; Carrington, 1979; Fontaine and Hoadley, 1976; Grabow et al., 1976; Peou et al., 1981; Sturtevant and Feary, 1969). Many of the investigations into the selectivity of wastewater treatment for antibiotic-resistant bacteria had insufficient or inadequate data from which to draw statistically significant conclusions.

Given the large degree of variation in wastewater quality, a large number of observations are required to reach statistically valid conclusions. Statistically designed experiments can help overcome some of the problem (Box et al., 1978). A further complication arises from lack of independence amongst the samples obtained from wastewater effluents. All parametric and nonparametric tests assume that the samples are randomly obtained from a population and that each sample is independent. Serial correlation such as arises from sampling continuous processes can alter the assumption of independence and lead to erroneous conclusions (Berthouex et al., 1978; Box et al., 1978).

An additional problem with earlier work on the topic was the use of the coliform group of bacteria as the test organisms. Several fæcal and non-fæcal genera of bacteria are included in the coliform definition (Feachem et al., 1983). Consequently, the response to antibiotics by coliforms is actually a confusion of the responses by the individual genera present. Thus the natural variation in the population mix can increase the variability observed in the antibiotic response.

The objective of this study was to compare the frequency of occurrence of MAR E. coli in the effluent of an activated sludge plant with their occurrence in the raw wastewater, to determine if the activated sludge process promotes antibiotic-resistance among fæcal coliform indicator bacteria.

2.2 MATERIALS AND METHODS

2.2.1 Wastewater Sampling

Wastewater samples were collected from the influent and effluent of the Gold Bar Wastewater Treatment Plant which serves the City of Edmonton, Alberta. Average daily flow rates are in the order of 250 ML/d. Thirty minute composite samples were collected at the plant headworks and outfall structure. Each sample was composited by withdrawing a 250 mL volume of wastewater every 3.7 min by an automatic sampler. The samples were analysed within one hour of collection. Sampling was done during April and May 1986.

2.2.2 Experimental Design and Analysis

The high average flow-rate into the plant, the sewer system retention time and backmixing in the plant were assumed to contribute to a relatively uniform daily bacteriological water quality. Also, it typically takes one week or less for antibiotic-resistant strains of bacteria to colonize the gut and these strains can be shed for two or three weeks after the antibiotic treatment is completed (Hardy, 1981). On this basis, it was assumed that the effluent population of MAR _E. coli_ was typical of the treated wastewater and that the influent MAR _E. coli_ population was typical of the raw sewage within a daily time period. However, since weekly and seasonal trends were likely, a pair-wise experiment was used to help eliminate variation introduced by these trends.

The paired Wilcoxon signed-rank test was used for data analysis since it provided a more conservative approach than the parametric Student's t-test which, though insensitive to deviations from Normality, was felt to be inadequate in this case where no information was available on the underlying frequency distribution (Davies, 1979; Dixon and Massey, 1969).

2.2.3 Isolation of _E. coli_ from Wastewater

Fæcal coliform bacteria were isolated from each wastewater sample using m-FC agar (Difco Laboratories, Inc.) and Method 909C (APHA, 1985). Following 24±2 h incubation at 44.5° C, 40 typical fæcal coliform colonies were selected at random and streaked on Tryptone Bile Agar (TBA; Oxoid Canada Inc.). It was assumed that 75% of fæcal coliforms were E. coli resulting in a minimum of 30 isolates for resistance testing. The TBA growth medium was incubated at 44.5° C for 24±4 h. An indole spot-test (Vracko and Sherris, 1963) was used to confirm indole production. Lactose fermentation and indole production were presumed to indicate E. coli (Anderson and Baird-Parker, 1975; Delaney et al., 1962). This was confirmed for 75, randomly selected presumptive E. coli isolates using the API 20E® System (API Laboratory Products Ltd.). All 75 isolates were confirmed as E. coli.

2.2.4 Antibiotic-resistance Testing

A typical presumptive E. coli was chosen from an indole positive TBA plate and transferred to Nutrient Agar (NA; Oxoid Canada Inc.) for a purity check. Concurrently, 5 mL of Tryptone Soya Broth (TSB; Oxoid Canada Inc.) was inoculated. Following overnight incubation (18 h) at 35° C, the TSB was diluted, 1 mL into 9 mL of sterile, buffered water (Bell et al., 1983). The suspension was split between two petri dishes, each containing 20 mL of Iso-Sensitest Agar (ISA; Oxoid Canada Inc.). Six different antibiotic disks (Oxoid Canada Inc.) were placed on each ISA plate using an Oxoid Mk II dispenser. The antibiotics used and their concentrations are given in Table 2.1. They were chosen according to their use for treating infections caused by members of Enterobacteriaceae (Abramowicz, 1982; Bauer et al., 1966; Joklik et al., 1980; NCCLS, 1983). The plates were incubated overnight at 35° C and scored for growth confluent with the disk (Bell et al., 1983). Those strains considered intermediate by standard definitions (Bauer et al., 1966; NCCLS, 1983) were designated as sensitive.

TABLE 2.1 Antibiotics used for screening E. coli isolated from raw influent wastewater and treated effluent.

Group	Antibiotic	Code	Dose (μg)
1. Aminoglycosides	Streptomycin	S	10
	Gentamicin	CN	10
	Kanamycin	K	30
2. Cephalosporins	Cephalothin	KF	30
3. Chloramphenicol	Chloramphenicol	C	30
4. Nitrofurans	Nitrofurantoin	F	300
5. Penicillins	Ampicillin	AMP	10
	Carbenicillin	CAR	100
6. Sulphonamides	Sulphafurazole	SF	300
	Sulphamethoxazole-trimethoprim	SXT	25
7. Tetracyclines	Tetracycline	TE	30
	Oxytetracycline	OT	30

2.3 RESULTS

The antibiotic-resistance score sheets for the E. coli isolates tested are provided in Appendix C. The proportions before and after activated sludge treatment were calculated on the basis of cumulative, multiple-resistances. That is, greater than or equal to one, greater than or equal to two, and so on. Table 2.2 summarizes the data for each of the test pairs. A total of 405 and 408 E. coli isolates from the raw influent wastewater and final effluent, respectively, were examined for antibiotic-resistance. Table 2.3 tabulates the observed number of strains resistant to individual antibiotics from the influent and effluent populations of E. coli. Table 2.4 presents the same information as Table 2.3 but categorizes resistances by antibiotic group (Table 2.1). The observed number of MAR strains with simultaneous resistance to 1,2,3 and so on antibiotics are summarized in Table 2.5. Table 2.6 tabulates the cumulative occurrence of MAR strains in the raw influent wastewater and the treated effluent. The significance level in Table 2.6 is the probability that there is not a greater proportion of MAR E. coli in the effluent when compared with the influent based on the 14 paired observations.

The geometric mean densities of fæcal coliform bacteria in the raw influent and final effluent were $10^{6.14}$ and $10^{4.29}$ CFU/dL, respectively for the 14 paired observations. The mean E. coli proportion of fæcal coliforms in the influent wastewater was 91% with a standard deviation of 8.2%. The mean E. coli proportion of fæcal coliforms in the final effluent was 88% and with a standard deviation of 8.6%.

2.4 DISCUSSION

It is clear from Table 2.6 that MAR E. coli constitute a significantly greater proportion in the final effluent of the activated sludge plant studied when compared with the influent wastewater. This suggests that the environment

TABLE 2.2 Summary of MAR E. coli proportions observed in the raw influent and treated effluent.

Date	Time		FC density, log CFU/dL	E. coli proportion of FC, %	Strains tested	Cumulative MAR greater than or equal to, %					
						1	2	3	4	5	6
22.04.86	0900	R	6.06	97	29	44.8	33.3	31.0	20.0	6.67	3.33
		SE	4.33	92	30	63.3	43.3	40.0	30.0	20.0	10.0
	1300	R	6.13	95	30	53.3	40.0	20.0	20.0	13.3	6.67
		SE	4.51	78	28	67.8	50.0	39.3	30.0	23.3	16.6
29.04.86	0900	R	5.86	88	30	23.3	10.0	6.67	3.33	3.33	0.00
		SE	3.91	[illegible]	30	43.3	33.3	26.7	23.3	10.0	3.33
	1300	R	6.08	88	30	53.3	40.0	30.0	20.0	3.33	3.33
		SE	3.85	65	26	57.7	53.8	46.2	42.3	26.9	19.2
06.05.86	0900	R	6.05	100	30	50.0	36.7	30.0	16.7	13.3	10.0
		SE	4.36	90	30	53.3	43.3	36.7	30.0	16.7	13.3
	1100	R	6.11	90	30	50.0	40.0	33.3	20.0	16.7	13.3
		SE	4.20	82	29	65.5	48.3	41.4	31.0	27.6	10.3
07.05.86	0900	R	6.04	78	28	50.0	25.0	17.9	7.14	7.14	3.57
		SE	4.30	93	30	60.0	36.7	33.3	20.0	13.3	10.0
	1030	R	6.15	92	24	54.2	29.2	29.2	20.8	12.5	4.17
		SE	4.28	85	25	68.0	44.0	24.0	12.0	0.00	0.00
	1230	R	6.32	78	28	39.3	28.6	21.4	14.3	7.14	3.57
		SE	4.04	88	30	60.0	36.7	33.3	26.7	13.3	6.67
	1400	R	6.26	93	30	63.3	40.0	26.7	20.0	3.33	0.00
		SE	4.49	90	30	76.7	36.7	33.3	30.0	20.0	10.0
13.05.86	0900	R	6.16	95	30	80.0	56.7	33.3	23.3	20.0	16.7
		SE	4.43	90	30	70.0	50.0	36.7	30.0	23.3	16.7
	1100	R	6.18	98	30	96.7	60.0	30.0	30.0	20.0	6.67
		SE	4.53	95	30	76.7	66.7	56.7	46.7	43.3	16.7
	1300	R	6.19	95	30	66.7	33.3	23.3	20.0	10.0	6.67
		SE	4.38	100	30	96.7	60.0	43.3	40.0	26.7	13.3
	1430	R	6.34	95	26	80.8	53.8	38.5	34.6	19.2	15.4
		SE	4.45	93	30	83.3	63.3	40.0	23.3	10.0	3.33
				Ranked-sign statistic		89_{+}	101_{+}	102_{+}	96_{+}	90_{+}	73_{+}

R = raw wastewater SE = secondary effluent FC = fæcal coliforms.
405 strains from influent and 408 strains from effluent were tested

TABLE 2.3 Number of E. coli strains observed to be resistant to each antibiotic irrespective of single or multiple-resistances.

Antibiotic†	Influent	Effluent	$\frac{\text{Effluent}}{\text{Influent}}$
S	80	117	1.46
CN	4	5	1.25
K	28	41	1.46
KF	48	50	1.04
C	14	26	1.86
F	24	21	0.88
AMP	63	85	1.35
CAR	50	89	1.78
SF	140	178	1.27
SXT	32	32	1.00
TE	84	124	1.48
OT	96	133	1.39

405 strains from influent and 408 strains from effluent were tested
† See Table 2.1 for key to antibiotic codes

TABLE 2.4 Number of E. coli strains observed to be resistant to each antibiotic group irrespective of single or multiple-resistances.

Antibiotic group†	Influent	Effluent	$\frac{\text{Effluent}}{\text{Influent}}$
1	112	163	1.46
2	48	50	1.04
3	14	26	1.86
4	24	21	0.88
5	113	174	1.54
6	172	210	1.22
7	180	257	1.43

405 strains from influent and 408 strains from effluent were tested

† See Table 2.1 for antibiotic group code

TABLE 2.5 Number of individual E. coli strains with simultaneous multiple-resistances in the raw influent and treated effluent.

Number of simultaneous resistances	Influent	Effluent	Effluent/Influent
2	41	38	0.93
3	33	36	1.09
4	32	41	1.28
5	18	36	2.00
6	18	14	0.78
7	3	21	7.00
8	5	3	0.60
9	1	5	5.00

405 strains from influent and 408 strains from effluent were tested

TABLE 2.6 Cumulative totals of E. coli strains with simultaneous multiple-resistances in the raw influent and treated effluent.

Number of simultaneous resistances	≥1	≥2	≥3	≥4	≥5	≥6
Influent	235	151	110	77	45	27
Effluent	272	184	156	120	79	43
$\frac{\text{Effluent}}{\text{Influent}}$	1.16	1.27	1.37	1.53	1.78	1.63
Significance level of difference†	**	**	**	**	**	*

† Based on observations in Table 2.2.

* Pr≤0.05 ** Pr≤0.01

of an activated sludge plant favours the selection of MAR E. coli. Sagik et al. (1981) studied the survival of plasmid-bearing E. coli in a bench-scale activated sludge plant. They could not find evidence to support the hypothesis that the presence of a plasmid in the cell conferred differential survivability. Peou et al. (1981) concluded that no statistically significant difference was apparent in the proportion of antibiotic-resistant bacteria when comparing influent and effluent proportions in an activated sludge treatment plant treating 150 ML/d of wastewater.

There are several possible explanations for the conflicting reports in the literature. An important question is the mechanism by which the proportion of MAR bacteria increase in the effluent. One explanation is that the presence of a resistance plasmid confers differential survivability for the bacterium. Another explanation could be that the transfer of plasmids mediating resistance to antibiotic-sensitive strains could be significant in the treatment process leading to increased proportions of MAR bacteria in the final effluent. This phenomenon would be highly dependent on contact time and the presence of intermediate bacteria with conjugative plasmids as suggested by Sagik et al. (1981). They found that the introduction of an E. coli with plasmid-mediated resistance to tetracycline, carbenicillin, and chloramphenicol transmitted this resistance to indigenous E. coli in a bench-scale activated sludge plant. Addition of a plasmid-mobilizing strain of E. coli greatly increased the transfer frequency of the resistance plasmid.

Gealt et al. (1985) also found that a recipient strain of E. coli isolated from wastewater received a resistance plasmid from a donor strain when coincubated in sterile wastewater confirming that gene transfer is a viable process in wastewater. Mach and Grimes (1982) found significant plasmid transfer frequencies in the primary and secondary clarifiers of an activated sludge plant. Therefore, transfer of resistance plasmids may account for the increase in proportion of MAR E. coli observed in an activated sludge plant.

Grabow et al. (1976) reported an increase in the proportion of MAR coliforms with transferable resistance factors through a trickling filter plant with primary and secondary sedimentation, chlorination, and rapid sand filtration. They postulated that the quiescent conditions in the clarifiers were suitable for significant transfer of resistance plasmids.

Gold Bar Wastewater Treatment plant is a plug flow activated sludge process with typical pretreatment by screening and grit removal before the primary clarifiers. Unlike most municipal activated sludge plants, all waste sludge from the mixed liquor and the secondary clarifier is recycled to the primary clarifier where sludge is withdrawn and pumped to the sludge digestors. Quiescent conditions in an environment containing a high concentration of microorganisms are known to promote the transfer of resistance plasmids and may account for the significant increase in the proportion of MAR bacteria in the final effluent of this activated sludge plant (Gealt et al., 1985; Mach and Grimes, 1982).

Table 2.4 illustrates that E. coli were more frequently resistant to some groups of antibioitics, notably the tetracyclines, penicillins, aminoglycosides, and sulphonamides, than to others. This observation is dependent to a large extent on the choice of antibiotic used in the experiment. For instance, the latest generation of antibiotics such as amikacin (30 μg), tobramycin (10 μg), and cefoxitin (30 μg) were screened, but all were found to be consistently antimicrobial. Abramowicz (1982, 1986) and NCCLS (1983) outlined the principal antimicrobials used clinically. The University of Alberta dispensary indicated that the most frequently dispensed antimicrobials were erythromycins (≃35%), tetracyclines (≃30%), penicillins (≃20%), and sulphonamides (≃15%). This fact is reflected in Table 2.4 where E. coli were frequently resistant to the last three groups. Erythromycin-resistance was not investigated in this study. The Pharmacy Department of University of Alberta Hospitals estimated that cephalosporins and aminoglycosides were the most frequently used antibiotics (≈25% each) followed by penicillins (≈15%), and tetracyclines (≈5%). Sulphur drugs were rarely used. The frequency of aminoglycoside resistance in Table 2.4 likely reflects the use of these antibiotics in Edmonton hospitals.

Table 2.5 summarizes the number of individual MAR E. coli observed during the course of the study. Examination of Tables 2.5 and 2.6 suggests that E. coli resistant to several antibiotics simultaneously, generally occur more frequently in the final effluent than in the raw influent wastewater.

2.5 CONCLUSIONS

It was found in this study that the proportion of MAR E. coli in the final effluent of an activated sludge plant was greater when compared with the proportion in the raw influent wastewater. Two possible reasons for this observation are increased survivability of MAR E. coli compared with antibiotic-sensitive strains of E. coli, and the transfer of resistance plasmids during the treatment process. Regardless of the reasons for increased proportions of MAR bacteria in the final effluent, there is cause for concern regarding the large numbers of bacteria with potentially transferable antibiotic-resistance which are discharged into the receiving environment. Many pathogens are members of Enterobacteriaceae and the transfer of resistance plasmids between E. coli, Shigella spp., and Salmonella spp. is well documented (Hardy, 1981).

The density of fæcal coliform bacteria in sewage effluents from mechanical plants normally exceeds water quality guidelines for primary contact recreational use even after dilution. Therefore it is necessary to consider wastewater disinfection as a means of controlling the discharge of potentially harmful organisms into the environment where the water is reused.

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3. OZONE DISINFECTION OF SECONDARY EFFLUENT CONTAINING MAR E. COLI

3.1 INTRODUCTION

In Chapter 2 it was concluded that there was an increase in the proportion of MAR E. coli as a result of wastewater treatment using the activated sludge process. Wastewater disinfection is a control method for the prevention of microbiological pollution (Bell and Smith, 1982). Chlorine is the most widely used disinfectant in North America, however it has several disadvantages including poor performance, production of toxic chemical by-products and a residual which is toxic to aquatic organisms (Health and Welfare Canada, 1984; Feachem et al., 1983). Alternatives to chlorine include chlorine dioxide, bromine, iodine, ultraviolet radiation, and ozone (United States EPA, 1986; WPCF, 1986). A few studies have been undertaken to investigate the effects of disinfection on antibiotic-resistant indicator bacteria. Murray et al. (1984) found that chlorination of coagulated and settled raw wastewater selected for antibiotic-resistant, lactose-fermenting bacteria. Another study (Rusin, 1986), also found that faecal coliform bacteria resistant to two or more antibiotics were selected for by chlorination of secondary effluent. Armstrong et al. (1982) concluded that chlorination of drinking water supplies selected for MAR heterotrophic bacteria. Meckes (1982) investigated the survival of antibiotic-resistant coliforms isolated from a filtered, ultraviolet disinfected activated sludge effluent. He found that the proportion of the total coliform bacteria resistant to tetracycline and chloramphenicol increased in the irradiated effluent.

Very few reports were found in the literature concerning ozonation of antibiotic-resistant bacteria. Yakovleva and Il'nitskii (1967) studied ozonation of antibiotic-resistant Shigella and Escherichia in water but did not examine the

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problem of differential selection. One unpublished study prepared for the United States EPA concluded that chlorine, ozone, and ultraviolet radiation did not select for antibiotic-resistant bacteria during secondary effluent disinfection (Staley, 1985).

The goal of the study reported here was to ascertain if ozone differentially selected for MAR E. coli when disinfecting an activated sludge effluent.

3.2 EXPERIMENTAL PROTOCOL

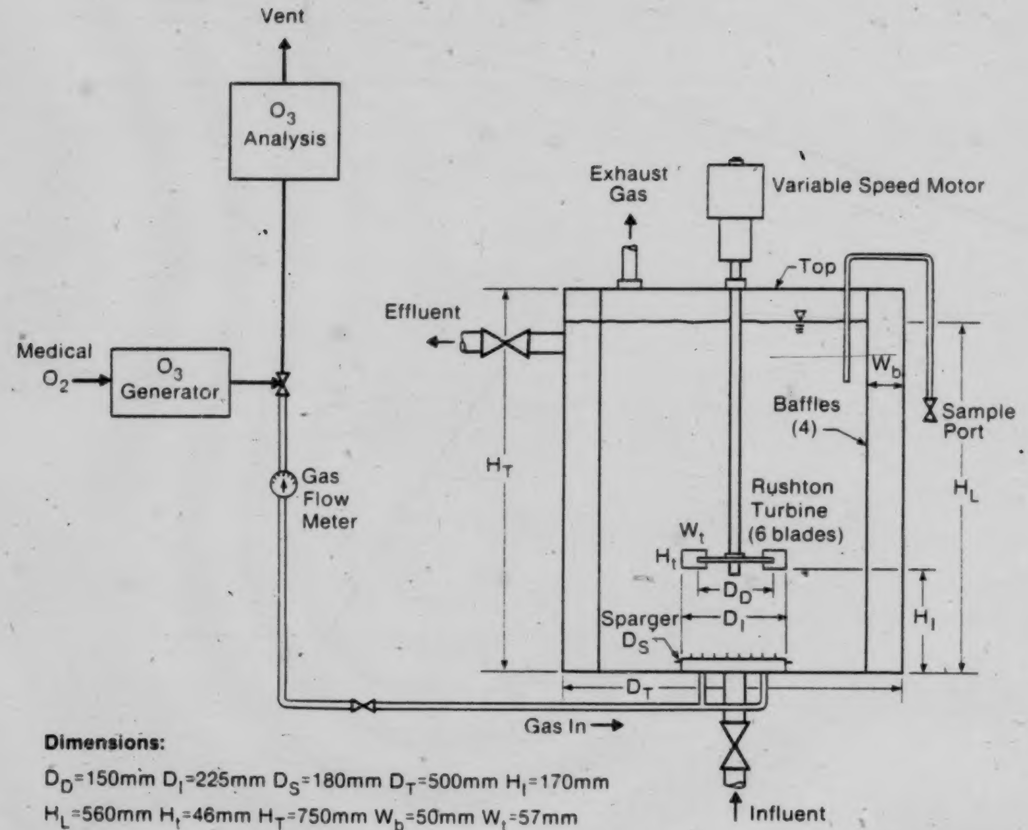
3.2.1 Ozone Equipment and Analytical Methods

Ozone was generated from medical grade oxygen using a corona discharge ozone generator (Model C2P-9C-4; PCI Ozone Corp.). The 110 L semi-batch stirred tank gas-liquid reactor (BSTR) described by Prengle et al. (1976). The impeller was a 6-blade Rushton turbine (Rushton et al., 1950a, 1950b). The detailed design of the reactor and the shop drawings are provided in Appendix A. Figure 3.1 illustrates the details of the reactor design and a schematic layout of associated valves and piping.

3.2.2 Wastewater Sampling

Secondary effluent from the City of Edmonton's Gold Bar Wastewater Treatment Plant was used for the disinfection trials. Daily composite wastewater characteristics were determined by the City of Edmonton's laboratory using standard methods (APHA, 1985). The values of selected water quality parameters for the dates of the experiments are tabulated in Table 3.1. All bacteriological samples were collected according to Method 906A (APHA, 1985). Ozonated samples were treated with excess 1% sodium thiosulphate to neutralize residual oxidants. Samples were stored on ice and in the dark for 2 h or less prior to analysis.

FIGURE 3.1 Schematic layout of ozonation apparatus and details of the semi-batch stirred tank reactor design (after Prenglé et al., 1976).


Vent
O_3 Analysis
Exhaust Gas
Variable Speed Motor
Top
Effluent
Medical O_2
O_3 Generator
W_b
Baffles (4)
Sample Port
H_T
Gas Flow Meter
Rushton Turbine (6 blades)
H_L
W_t
H_t
D_D
Sparger
D_I
H_I
D_S
D_T
Gas In
Influent

Dimensions:

D_D=150mm D_I=225mm D_S=180mm D_T=500mm H_I=170mm

H_L=560mm H_t=46mm H_T=750mm W_b=50mm W_t=57mm

Sparger hole density:

4650 — 1.5 mm ϕ holes per m^2

Materials:

316 Stainless Steel and Teflon

TABLE 3.1 Characteristics of the wastewater on the day of the disinfection experiment based on 24 h composite samples collected and analysed by the City of Edmonton.

Characteristic†		Sampling date				
		12.06.86	17.06.86	18.06.86	23.06.86	24.06.86
COD,	mg/L	55	35	43	32	52
BOD_5,	mg/L	21	22	20	27	48
TSS,	mg/L	27	6	8	7	10
NH_3-N,	mg/L	—	—	9.7	9.0	—
NO_3^--N,	mg/L	—	—	4.8	7.8	—
PO_4^{3-}-P,	mg/L	—	—	3.5	3.2	—
Temperature,	°C	19	18	19	19	19

† According to standard methods APHA (1985)

COD = chemical oxygen demand

BOD_5 = 5-day biochemical oxygen demand

TSS = total suspended solids

3.2.3 Experimental Design and Analysis

The most efficient experimental design for comparing the difference between two treatments is a paired design where before and after observations are obtained using the same batch of material (Box et al., 1978). Paired observations eliminate the effect of batch-to-batch variation in wastewater and non-Normal distributions have a reduced impact on the attained significance level (Davies, 1979). However, every effort must be made to randomize the samples of wastewater since lack of independence in the observations can adversely affect the attained significance levels (Box et al., 1978).

In this experiment, batches of secondary effluent were examined for the proportion of MAR E. coli before and after treatment with ozone. The paired observations were analysed using the nonparametric Wilcoxon signed-rank test (Dixon and Massey, 1969). This procedure also provides a more conservative estimate of the significance levels than parametric procedures, such as the paired Student's t-test (Davies, 1979).

3.2.4 Procedure

The BSTR was filled with 110 L of secondary effluent. A 500 mL grab sample was taken from the mixed reactor contents for determination of the MAR E. coli proportion before ozone treatment. The ozone generator was run for 5 min prior to starting a run to ensure that the generator output had stabilized. Ozone concentration in the carrier gas was determined before and after each experiment and the average of the two concentrations was used to calculate the applied ozone dose. Gas flow-rate was calibrated using a wet test meter (Precision Scientific, Inc.).

The required ozone dose to achieve significant fæcal coliform reduction was estimated from a model developed by Caverson et al. (1986) for primary effluent from the same plant:

$$\log\frac{N}{N_0} = -6.68 - 3.95\ \log C_A + 5.50\ \log BOD_5 \qquad [3.1]$$

where N is the concentration of surviving bacteria, N_0 is the initial concentration of bacteria, C_A is the applied ozone dose and BOD_5 the 5-day biochemical oxygen demand. The calculated applied dose for 99.9% fæcal coliform reduction was approximately 8.0 mg/L.

Ozone contact time in the reactor was 20 min after which time a 500 mL sample of ozonated effluent was obtained for subsequent antibiotic-resistance testing to determine the proportion of MAR E. coli in the treated effluent.

The concentration of ozone in the gas phase was determined using the potassium iodide procedure recommended by PCI Ozone Corp. which was based on the work of Birdsall et al. (1952). Reagents were prepared and standardized in accordance with Method 408A (APHA, 1985). Gas volumes were measured using the wet test meter and were reverted to standard temperature and pressure (STP; 273.15° K and 101.33 kPa). The applied ozone dose was calculated from the mass of ozone (at STP) applied to the 110 L volume of wastewater in the BSTR. Under the conditions of the experiment, it was determined through measurement of the off-gas ozone concentration, that greater than 96% of the applied ozone was transferred to the wastewater. Residuals were believed to be small on the basis of the work of Caverson et al. (1986). This assumption was supported by the residual ozone determinations in Chapter 7. Therefore, it was assumed that, for practical purposes, the ozone residual in the wastewater was at equilibrium with the ozone concentration in the head-space of the reactor.

Microbiological analysis and subsequent antibiotic-resistance testing followed procedures identical to those described in Chapter 2. However, the number of antibiotics tested was reduced from 12 to 11 because of an unexplained, virtually universal resistance by the E. coli strains examined to sulphafurazole. This anomoly was not observed previously nor in subsequent experiments and it greatly influenced the reduction of the data. Therefore, it was dropped from further analysis.

3.3 RESULTS

Raw scores for the antibiotic-resistance testing are provided in Appendix D. The tabulated proportions in Table 3.2 were obtained from the cumulative observed multiple-resistances. That is, bacteria with resistances to one or more antibiotics, two or more antibiotics and so on. The nonparametric Wilcoxon signed-rank test was used to evaluate the difference between the paired proportions of ozonated and non-ozonated MAR E. coli presented in Table 3.2. In all MAR classes, there was no evidence to support the hypothesis that there was a difference between the before and after proportions of MAR E. coli ($Pr \leq 0.05$).

In Table 3.3, the number of E. coli isolates which were resistant to each antibiotic irrespective of single or multiple resistances is summarized. Table 3.4 is a compilation of the number of individual MAR E. coli observed in the study. The proportions are of the number of MAR E. coli in each MAR class compared with the total number of isolates examined. There were 201 and 205 isolates of E. coli tested from the non-ozonated and ozonated secondary effluents, respectively.

3.4 DISCUSSION

Contrary to the findings of Murray et al. (1984) and Meckes (1982) for chlorine and ultraviolet radiation, respectively, ozone does not appear to select for MAR E. coli. There were a large number of isolates which were resistant to tetracyclines and penicillins in Table 3.3. As discussed in Chapter 2, these two were among the most commonly prescribed groups of antibiotics at the University of Alberta dispensary and a major pharmacy. Little selective pressure was

TABLE 3.2 Summary of applied ozone doses and proportions of MAR E. coli before and after ozone treatment.

Date	Time		Applied ozone dose, mg/L	FC density, log CFU/dL	E. coli proportion of FC, %	Strains tested	Cumulative MAR greater than or equal to, %				
							1	2	3	4	5
12.06.86	1200	SE		5.04	84.2	30	60.0	40.0	20.0	13.3	10.0
		OZ	9.8	2.15	92.5	29	65.5	48.3	34.5	27.6	3.45
17.06.86	1200	SE		4.98	82.5	30	80.0	70.0	46.7	33.3	30.0
		OZ	8.6	2.41	95.0	30	40.0	36.7	20.0	10.0	3.33
	1300	SE		4.92	92.5	30	23.3	23.3	10.0	3.33	0.00
		OZ	8.4	2.03	95.0	27	55.6	40.7	22.2	7.41	7.41
18.06.86	1115	SE		4.81	90.0	30	46.7	40.0	23.3	6.67	3.33
		OZ	8.4	2.42	87.5	30	56.7	40.0	30.0	26.7	23.3
23.06.86	1445	SE		4.83	92.5	30	60.0	33.3	23.3	10.0	3.33
		OZ	8.8	2.30	97.5	30	40.0	30.0	10.0	3.33	3.33
24.06.86	0915	SE		4.99	96.0	24	45.8	41.7	29.2	12.5	8.33
		OZ	8.4	2.37	88.6	29	41.4	37.9	31.0	17.2	13.8
	1015	SE		4.80	93.1	27	44.4	37.0	25.9	18.5	14.8
		OZ	8.4	2.40	97.1	30	36.7	36.7	23.3	23.3	13.3
					Ranked-sign statistic		16_-	12_-	14_+	17_+	11_+

SE = secondary effluent OZ = ozone treated effluent FC = fæcal coliforms
201 strains from untreated effluent and 205 strains from treated effluent were tested

TABLE 3.3 Number of occurrences of resistance to each antibiotic irrespective of single or multiple-resistances.

Group†	Antibiotic†	Secondary effluent	Ozonated effluent	Treated / Untreated
1	S	37	53	1.4
	CN	6	2	0.33
	K	19	21	1.1
2	KF	20	19	0.92
3	C	8	7	0.88
4	F	6	9	1.5
5	AMP	34	25	0.74
	CAR	32	27	0.84
6	SXT	16	4	0.25
7	TE	65	61	0.94
	OT	66	64	0.97

201 strains from untreated effluent and 205 strains from treated effluent were tested

† See Table 2.1 for key to anitibiotic codes

TABLE 3.4 Distribution of E. coli strains which had simultaneous multiple-resistances.

Number of simultaneous resistances	Secondary effluent, % (201 isolates)	Ozonated effluent, % (205 isolates)	$\frac{\text{Treated}}{\text{Untreated}}$
1	8.5	9.3	1.1
2	15.6	13.4	0.87
3	12.0	8.8	0.73
4	4.0	7.3	1.8
5	6.0	5.4	0.90
6	3.0	1.0	0.33
7	1.0	2.0	2.0
8	0.5	0.5	1.0
Sub-total	51	48	0.94
0	49	52	1.1
Total	100	100	

associated with any one antibiotic group though E. coli with sulphamethoxazole-trimethoprim resistance seemed to be more affected by ozone than E. coli with other antibiotic-resistance patterns. Streptomycin resistance appeared to be more common in the ozonated effluent. There were too few observations of gentamicin and nitrofurantoin resistance to reach a valid conclusion. Furthermore, examination of individual resistances has little practical value since each generation of antibiotic produces different responses from the bacteria. Of more interest are the trends associated with presumed plasmid-mediated resistance and its interaction with the disinfectant. The ratio of the number of individual MAR E. coli observed in each MAR class, $\frac{\text{treated}}{\text{untreated}}$, illustrates the lack of overall differential selectivity (Table 3.4). However, beyond observations of simultaneous resistance to three antibiotics, there were too few isolates to properly evaluate trends.

The differences in the reported selectivity of MAR bacteria in disinfected water may arise from differences in the mode of action of the various water disinfectants. The United States NRC (1980) summarized the modes of action of free and combined chlorine, ultraviolet radiation and ozone. They noted that information to that date indicated that free chlorine and ozone were believed to inactivate cells through oxidation of enzyme systems and the unsaturated bonds of fatty acids in the cell membrane, respectively. Other evidence suggests that ozone also acts on cell DNA (Hamelin and Chung, 1974; Ishizaki et al., 1987). The U.S. review also reported that ultraviolet radiation and chloramines may affect cell DNA (Fetner, 1962; Shih and Lederberg, 1976). Day (1982) noted that resistance plasmids may have genes which produce enzymes which repair damaged DNA, therefore it may be possible that resistance plasmids have a role in resistance to disinfectants. More investigation of this topic is needed.

3.5 CONCLUSIONS

The data reported here indicate that ozone disinfection of wastewater containing MAR E. coli does not differentially select these bacteria, as has been reported for chlorine and ultraviolet radiation. This finding further supports the need for alternative disinfection methods to chlorine and demonstrates the potential of ozone as an alternative to chlorine for wastewater disinfection. It was postulated that the reason that ozone was non-selective for MAR E. coli was that its mode of action was not oriented towards the genetic components of the cell but rather at the structural components of the cell wall. Further research into genetically-mediated resistance to disinfectants is needed.

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4. DOSE-RESPONSE OF FÆCAL COLIFORMS AND MAR E. COLI DURING OZONE DISINFECTION OF ACTIVATED SLUDGE EFFLUENT

4.1 INTRODUCTION

In Chapter 3, it was found that ozone did not differentially select for MAR E. coli when using an applied dose sufficient to remove approximately 99.9% of the fæcal coliform population. However, during a test run with the apparatus, only one quarter of the target dose was applied. It was observed that there was a larger proportion of MAR E. coli in the disinfected effluent compared with the untreated effluent. This observation raised the possibility that there may be a relationship between the proportion of MAR E. coli in the wastewater and the applied dose of ozone. No reports have been found in the literature regarding ozone disinfection of MAR E. coli using a range of applied ozone doses. Consequently, this study was intended to provide information on the behaviour of MAR E. coli at different doses of ozone.

A secondary objective was the tentative determination of the fæcal coliform dose-response function which could be compared with other models reported for secondary effluents. Caverson et al. (1986) discussed several of the published ozone dose-response models and noted that there were potential deficiencies in the experimental methodology and that the model parameters varied widely. Rakness et al. (1986) speculated on the reasons for the apparent differences between studies and between pilot-scale and full-scale plants. They thought that differences in mixing, short-circuiting, and retention time accounted for the variability in the responses.

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4.2 EXPERIMENTAL PROTOCOL

4.2.1 Microbiological Procedures

The microbiological analyses were identical to those used in Chapter 2. All of the antibiotics used in Chapter 2 were used in this experiment (see Table 2.1).

However, with respect to enumeration of bacteria by membrane filtration (MF), Haas and Heller (1986) discussed the statistical basis of the MF procedure and described tests used to determine if the underlying assumptions were valid. It was decided to implement a diagnostic check on the procedure using a method first described by Fisher et al., (1922) and reported by Haas and Heller (1986). The test consists of performing replicate analyses on the same sample and then checking the assumption that the probability distribution of the colony forming units on the plate is Poissonian using the Chi-squared goodness-of-fit test. This diagnostic test permits the analyst to check the MF method for estimating the coliform bacteria density in water. If the variance of replicate analyses exceeds that expected for a Poisson distribution, then there is reason to suspect that there may be a problem with the analytical method and that the results should be viewed with scepticism.

4.2.2 Ozone Equipment and Analytical Methods

The ozone apparatus and analytical techniques were identical to those reported in Chapter 3. Figure 3.1 described the the semi-batch stirred tank (BSTR) reactor used in this experiment.

Ozone concentration in the gas phase was determined using an ultraviolet spectrophotometer (Model HC-12; PCI Ozone Corp.). The monitor was periodically checked using the iodometric method of Birdsall et al. (1952), as recommended by the manufacturer. Applied ozone dose was calculated from:

$$C_A = \frac{C_g \cdot Q_g \cdot t_g}{V} \qquad [4.1]$$

where C_A is the applied dose, C_g is the concentration of ozone in the gas phase (STP), Q_g is the gas flow-rate (STP), t_g is the ozonation time and V is the volume of wastewater treated.

4.2.3 Wastewater Sampling

A fresh batch of secondary effluent was obtained for each dose-response trial from the City of Edmonton's Gold Bar Wastewater Treatment Plant. The wastewater characteristics were determined from 24 h composite samples analysed by the City of Edmonton's laboratory using standard methods (APHA, 1985). The values of selected water quality parameters for the day of the trial are tabulated in Table 4.1.

4.2.4 Procedure

The applied ozone doses were an approximate geometric progression from 0 to 10 mg/L since the response function was expected to be lognormally distributed (Finney, 1978). The doses applied to the reactor were determined by calculating the applied mass of ozone per unit time per unit volume and withdrawing 400 mL samples at the appropriate time intervals. The resulting applied dose for each trial is tabulated in Table 4.2. The samples which were removed from the reactor were left at the temperature of the wastewater and in the dark for up to 20 min to ensure that all samples were treated equally. Starting time was at the start of gas flow to the reactor and 20 min later each sample had any residual oxidants neutralized with excess 1% sodium thiosulphate. Samples were immediately placed in a crushed-ice water bath and kept in the dark. They were analysed within 2.5 h for fæcal coliforms.

TABLE 4.1 Characteristics of the wastewater on the day of the disinfection experiment based on 24 h composite samples collected and analysed by the City of Edmonton.

Characteristic†		Sampling date Trial 1 15.07.86	 Trial 2 & 3 11.08.86
COD,	mg/L	36	53
BOD_5,	mg/L	31	33
TSS,	mg/L	8	18
PO_4^{3-}-P,	mg/L	3.6	3.1
pH		7.8	7.8
Total alkalinity, (as $CaCO_3$)	mg/L	150	140
Temperature,	°C	19	20

† According to standard methods APHA (1985)

COD = chemical oxygen demand

BOD_5 = 5- day biochemical oxygen demand

TSS = total suspended solids

TABLE 4.2 The observed responses of fæcal coliform bacteria and MAR E. coli to various applied ozone doses in secondary effluent.

Date	Trial	Applied ozone dose, mg/L	FC density, log CFU/dL	E. coli proportion of FC, %	Strains tested	Cumulative MAR greater than or equal to, %		
						3	4	5
15.07.86	1	0.00	4.36*	91.9	30	16.7†	13.3†	10.0†
		0.39	4.18	93.8	28	25.0	17.9	10.7
		0.98	4.15	91.9	30	46.7	26.7	16.7
		1.97	4.15	89.2	28	46.4	35.7	25.0
		4.55	2.60	56.8	19	26.3	15.8	5.26
		9.95	1.15	41.7	10	10.0	10.0	10.0
11.08.86	2	0.00	3.76*	92.0	29	24.1†	20.7†	17.0†
		0.59	3.84	81.8	30	13.3	10.0	3.33
		1.16	3.54	96.0	29	17.0	10.3	10.3
		2.12	3.59	85.4	30	23.3	6.67	3.33
		5.19	2.69	92.0	30	30.0	20.0	16.7
		9.97	1.83	92.0	40	12.5	5.00	2.50
	3	0.00	3.69*	94.0	30	23.3†	16.7†	10.0†
		0.46	3.38	98.0	30	36.7	20.0	10.0
		0.92	3.45	94.0	30	16.7	10.0	6.67
		1.72	3.41	98.0	30	23.3	13.3	10.0
		4.12	2.51	88.9	30	13.3	6.67	6.67
		8.08	1.69	96.0	40	45.0	17.5	10.0

† P_o, initial proportion of MAR E. coli • N_o, initial fæcal coliform density FC = fæcal coliform

4.3 RESULTS

The variance of the replicate fæcal coliform analyses did not significantly exceed that expected for a Poisson distribution ($Pr \leq 0.05$). The results of the fæcal coliform and antibiotic-resistance analyses are summarized in Table 4.2. The antibiotic-resistance score sheets are provided in Appendix E. Figure 4.1 presents the fæcal coliform dose-response data using the dose and response metameters recommended for the design of ozone wastewater disinfection processes (United States EPA, 1986). The proportion of E. coli having simultaneous resistance to three or more, four or more, five or more antibiotics were designated as $MAR_{\geq 3}$, $MAR_{\geq 4}$, and $MAR_{\geq 5}$, respectively. To facilitate comparison between trials, the $\frac{P_i}{P_0}$ ratio was developed where P_i is the proportion of MAR E. coli observed at dose i and P_0 is the proportion of MAR E. coli before ozonation. The cumulative distribution function for each MAR class was used to estimate the median $\frac{P_i}{P_0}$ ratio for each class. The medians for $MAR_{\geq 3}$, $MAR_{\geq 4}$, and $MAR_{\geq 5}$ were, respectively, 1.0, 0.97, and 1.0. Figure 4.2 is a scatter plot of the $\frac{P_i}{P_0}$ ratios for all of the observations.

4.4 DISCUSSION

4.4.1 MAR E. coli Dose-response

The two largest ozone doses in Trial 1 had too few E. coli isolates chosen for reliable estimates of MAR proportions. However, the other two trials had sufficiently large sample sizes. Trials 2 and 3 were considered with and without Trial 1, yet no discernable difference was noted in the random variation of the $\frac{P_i}{P_0}$ ratio shown in Figure 4.2. Consideration of the median values of the $\frac{P_i}{P_0}$ ratio for each class of cumulative MAR E. coli in conjunction with the scatter plot of Figure 4.2 suggests that the variation in the proportion of MAR E. coli is a random occurrence and does not depend upon the ozone dose level.

FIGURE 4.1 Faecal coliform survival as a function of applied ozone dose in secondary effluent.

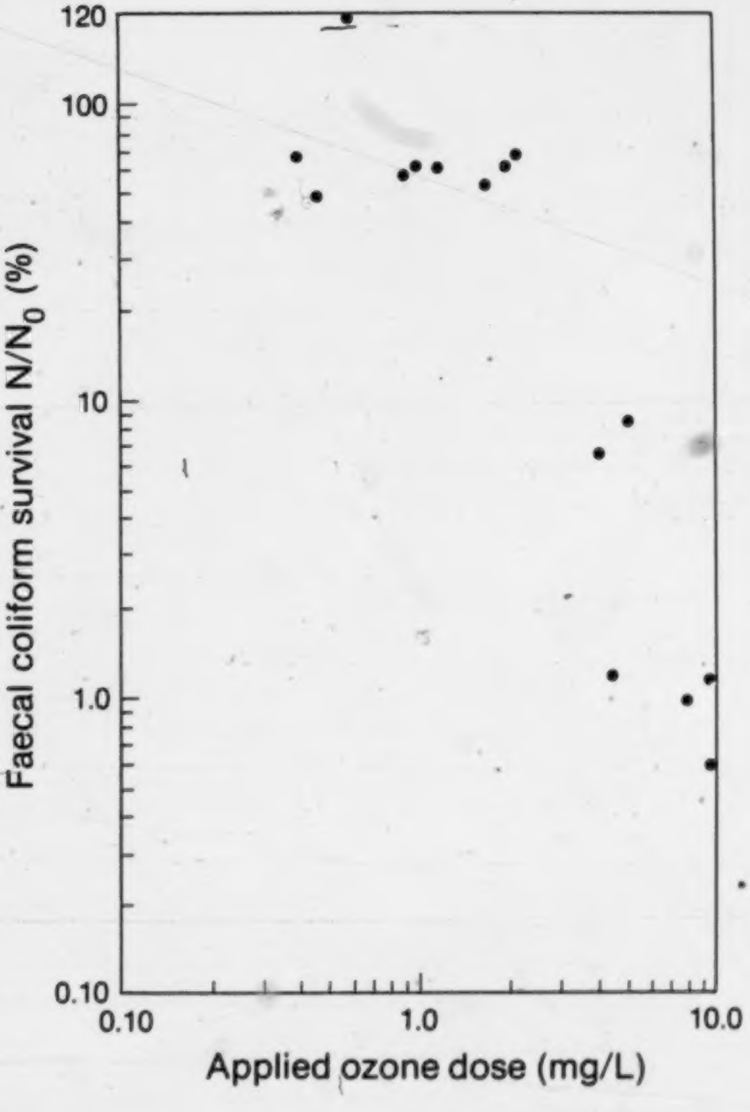
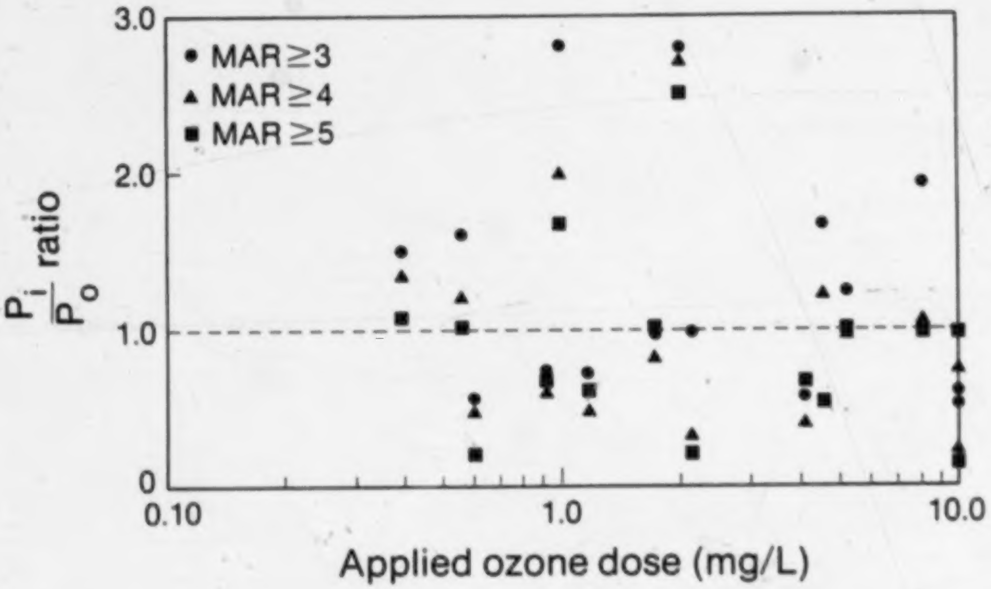


FIGURE 4.2 Scatter plot of the ratio of proportions of MAR E. coli before and after ozonation of secondary effluent at various doses of ozone.



These findings were consistent with those reported in Chapter 3, where it was concluded that no differential selectivity of MAR E. coli was associated with ozone disinfection of wastewater.

4.4.2 Fæcal Coliform Dose-response

Figure 4.1 is a scatter plot of the fæcal coliform disinfection data using the dose and response metameters commonly reported in the literature (Caverson et al., 1986; Given and Smith, 1979; Rakness et al., 1985). The form of the model is:

$$\log\frac{N}{N_0} = \beta_0 + \beta_1 \log C_{ozone} \qquad [4.2]$$

where N is the concentration of surviving bacteria, N_0 is the initial concentration of bacteria, C is the ozone dose, and β_0 and β_1 are model parameters.

The breakpoint or "threshold" dose, at about 1.8 mg/L in this case, is typical of ozone disinfection of wastewater where there was an initial ozone demand resulting from components in the wastewater which have much faster reaction rates than the disinfection reaction (Hoigńe, 1982; Smith 1967). The parameters for model [4.2] have typically been estimated by regression analysis of the doses and responses beyond the "threshold" dose (Caverson et al., 1986; Given and Smith, 1979; Meckes et al., 1983).

Using the fæcal coliform data from this study (Table 4.2, Figure 4.1), the parameters for [4.2] were estimated using the method of least-squares resulting in the following model:

$$\log\frac{N}{N_0} = 0.680 - 3.12 \log C_A \qquad [4.3]$$

Assuming that the model was adequate, the individual 95% confidence limits for the model parameters are:

$$-0.0170 \leq \beta_0 \leq 1.53$$

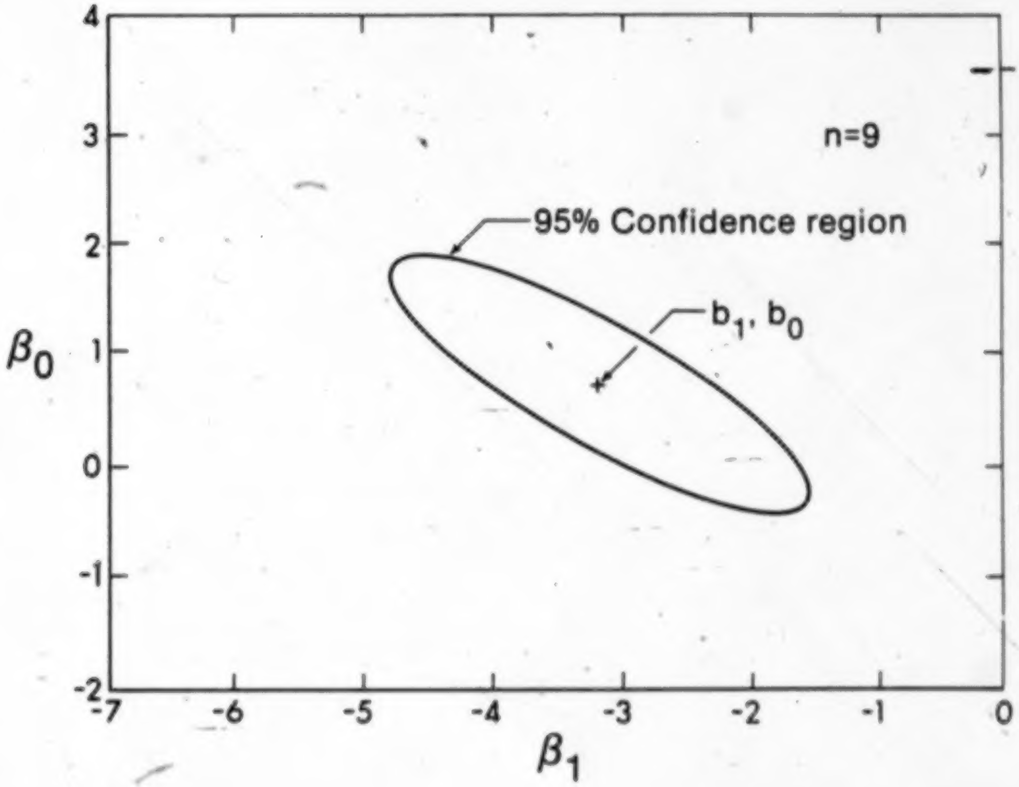
$$-4.34 \leq \beta_1 \leq -1.90$$

The 95% joint confidence region for the model parameters is plotted in Figure 4.3. The elliptical shape is typical of confidence regions where the estimates of the model parameters have very different standard errors. The inclination of the ellipse with respect to the major axis indicates that the parameter estimates are strongly correlated. Comparison with other parameter estimates reported in the literature should be done cautiously since the design of the experiment will influence the precision of the parameter estimates and the shape of the joint confidence region (Box et al., 1978; Draper and Smith, 1981).

Examination of residuals by means of a scatter plot revealed that the variance of the observations may not be constant from dose level to dose level. However, the limited degrees of freedom of the analysis did not permit adequate assessment of this factor. Caverson et al. (1986) could not find evidence of non-constant variance when evaluating ozone disinfection data from the same activated sludge plant as was used in this study. Similarly, analysis of the data collected by Given and Smith (1979) did not reveal any non-constant variance at the various dose levels. Therefore, for simplicity, model [4.3] will be assumed to be adequate and conforms to the underlying assumptions of the method of least-squares.

Comparison of the individual confidence limits of β_1 with parameter estimates reported by Caverson et al. (1986) and Rakness et al. (1986) suggests that there may be no statistically significant difference between parameter estimates associated with ozone dose (β_1). If this is the case, it is possible that the parameter estimate is only a function of the indicator bacteria being monitored during disinfection and that it may be independent of wastewater type or contactor design. However, when the two parameter estimates are considered jointly, it is apparent that the parameter estimates may be affected by the

FIGURE 4.3 95% joint confidence region for the dose-response model parameters, β_0 and β_1, obtained from the ozone disinfection data for fæcal coliforms in secondary effluent.



strength of the wastewater. For example, the data of Stover and Jarnis (1981) summarized by Rakness et al. (1985) indicate that the parameter estimates for ozone disinfection of total coliforms in wastewater with a COD of 40 mg/L lie within the joint confidence region of Figure 4.3. However, the same reactor with a lower strength wastewater, 20 mg/L COD, had data which provided parameter estimates which lay outside the joint confidence region.

Box et al. (1978) and Draper and Smith (1981) discuss in detail, the difficulties developing cause-effect models from happenstance data and suggest appropriate experimental methods for overcoming these problems. There is a need to evaluate the meaning of the dose-response relationship used for designing ozone wastewater disinfection processes. In particular, the relationship between the parameter estimates, microorganism type, the strength of the wastewater, and the type of contactor need to be investigated to provide a better understanding of ozone disinfection process for wastewater.

4.5 CONCLUSIONS

In Chapter 3 it was reported that ozone did not differentially select MAR E. coli when disinfecting effluent from an activated sludge plant. These findings were corroborated by the results of the dose-response experiments in this study in which no differential selectivity was observed, irrespective of dose level.

The fæcal coliform dose-response relationship plotted in Figure 4.1 demonstrated that a "threshold" dose exists when disinfecting wastewater with ozone. Modelling the response beyond the "threshold" dose produced parameter estimates which were similar in magnitude to those reported by others for the disinfection of secondary effluents. However, it was concluded that the parameter estimates may be a function of wastewater strength, organism type, and reactor type. Statistically designed experiments can help elicit information regarding the interactions between these components. Chapters 6 and 7 investigate the dose-response relationship between E. coli in ozone demand-free water and secondary effluent at bench and pilot-scales.

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5. EVALUATION OF THE STANDARD MEMBRANE FILTRATION PROCEDURE FOR THE RECOVERY OF A MARKER STRAIN OF OZONE INJURED E. COLI

5.1 INTRODUCTION

The problem of sub-lethal injury in fæcal coliforms was not considered in the previous chapters where MAR E. coli were investigated since it was not relevant to the recovery of wild strains of E. coli for antibiotic-resistance testing. Some of the problems with comparing the dose-response models for ozone disinfection of fæcal coliforms were discussed. Differences in ozone contactor type, mixing conditions, and hydraulic short-circuiting were suggested as the main reasons for the differences reported in the literature. It was also postulated that the parameter estimate associated with ozone dose may only be a function of the indicator organism being monitored during the disinfection process. In a model where only one independent variable is investigated, the parameter estimate associated with that variable is the slope (β_1). The slope describes the log unit reduction of bacteria per log unit increase in ozone dose (Figure 4.1 and model [4.2]). Several investigators have found that standard methods (APHA, 1985) for the recovery of coliform bacteria from stressed environments, such as disinfected waters and effluents, significantly underestimated the number of surviving, viable bacteria (Bissonnette et al., 1975, 1977; Camper and McFeters, 1979; LeChevallier et al., 1983, 1984; Lin, 1976; McFeters et al., 1982, 1986; Stuart et al., 1977). Intuitively, the degree of injury probably increases with the degree of disinfection. Therefore, it is possible that standard methods progressively underestimate the number of surviving organisms as the degree of disinfection increases. Consequently, dose-responses such as those shown in Figure 4.1, may have slopes which are apparently steeper or more variable simply because of the

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bacteriological methods. Therefore, it is important to know that the bacteriological method used for enumerating bacteria in a disinfected water is capable of accurate and precise estimation.

Several modifications have been proposed for improving the recovery of stressed fæcal coliforms by membrane filtration (MF) including elimination of rosolic acid from m-FC agar (Presswood and Strong, 1978), the use of a two-temperature incubation procedure (Green et al., 1977), and the use of various resuscitation media to allow growth of injured cells (Lin, 1976; Rose et al., 1975; Stuart et al., 1977). LeChevallier et al. (1983, 1984) evaluated m-T7 agar for recovery of injured total and fæcal coliforms and found it recovered significantly more fæcal coliforms than the standard methods (APHA, 1985).

The recovery of chlorine-stressed coliforms has been well documented (Cam~~per and McFeters, 197~~9; Green et al., 1977; Presswood and Strong, 1978; Stuart et al., 1977). One study which investigated the density estimates of ozonated coliforms from wastewater using the most probable number (MPN) and MF procedures concluded that there was no statistically significant difference between these methods (Meckes and Venosa, 1978).

This investigation had two purposes:

1. to develop a marker strain of typical fæcal coliform which could be seeded into batches of water containing mixed populations of microorganisms and yet be recovered without confusion with other indigenous organisms; and
2. to test the standard membrane filtration procedure for recovery of ozone injured organisms since it has been shown that significant underestimation of surviving bacteria can occur using standard procedures.

5.2 MATERIALS AND METHODS

5.2.1 Cultures

Escherichia coli strain 11775 from the American Type Culture Collection (ATCC®) was used to obtain a nalidixic acid resistant (Nal^R) mutant strain. An overnight culture of the nalidixic acid sensitive (Nal^S) parent strain was grown in 5 mL tryptone soya broth (TSB; Oxoid Canada Inc.) at 35° C and inoculated, undiluted, onto nutrient agar (NA; Oxoid) containing 50 µg of nalidixic acid per mL (NA_{50}). Excess culture was removed with a pasteur pipet. The NA_{50} plates were incubated overnight at 35° C. Colonies were selected, subcultured in 5 mL of TSB and inoculated onto NA containing 100 µg of nalidixic acid per mL (NA_{100}) to obtain a Nal^R E. coli strain resistant to 100 µg of nalidixic acid per mL. The parent and Nal^R strains of E. coli ATCC® 11775 were inoculated onto API 20E® strips (API Laboratory Products Ltd.) for biochemical identification. Both strains were stored on NA slants at 4° C.

5.2.2 Ozone Apparatus

An ultraviolet spectrophotometer (Model HC 12; PCI Ozone Corp.) was used to monitor continuously the concentration of ozone in the gas phase. Ozone was produced at room temperature from extra dry oxygen by a corona discharge ozone generator (Model C2P-9C-4; PCI Ozone Corp.). The gas flow-rate was 144 L/h (STP) and there was a gauge pressure of 38 kPa at the generator. Generation under these conditions produced an ozone concentration in the carrier gas of 69.7 mg/L (STP). The ozone-oxygen gas mixture was bubbled for a minimum of 40 min through 400 mL of 20° C MILLI-Q® water (Millipore Corp.) in a 500 mL gas absorption flask. Ozone concentration in the MILLI-Q® water was determined using the potassium iodide method of Birdsall et al. (1952)

by transferring an accurately measured volume of stock solution to 400 mL of 2% potassium iodide solution at approximately pH 6.0 followed by titration with standardized 0.01 N sodium thiosulphate at pH 2. This is very similar to the APHA standard method for ozone residual, but without the purge and trap step (APHA, 1985). The concentration of the stock ozone solution ranged from 20 to 23 mg/L.

Any glassware which could come into contact with ozone, or with water that would contact ozone, was washed in phosphoric acid and rinsed twice with 85° C deionized water. Glassware was soaked in a strong ozone solution for one hour and was dried at room temperature.

An ozone demand-free, 0.05 M phosphate buffer solution (pH 6.9) was prepared using MILLI-Q® water. One litre of phosphate buffer was placed in a 2000 mL Erlenmeyer flask containing a Teflon®-coated magnetic stirring bar. The test flask was ozonated for 180 s followed by a minimum of 300 s contact time to satisfy any ozone demand. The flask was autoclaved at 121° C for 15 min to remove residual ozone and was allowed to cool to 20° C in a laminar flow hood prior to use.

Initially, 0.85% saline was used as the test solution. However, upon ozonation, the solution was extremely toxic to the E. coli seed. This problem was not as severe when phosphate buffer was added to the saline, and it disappeared altogether when only the phosphate buffer was used. Therefore, phosphate buffered water was used as the test solution.

An additional problem also manifested itself during the course of the experiments. Certain types of glass washing soap were found to leave extremely persistent residuals, even after several rinses with high purity water. Ozonation of these soap residues proved to be very toxic to the seed organism. Therefore, glass washing soap was discontinued early in the program.

5.2.3 Experimental Design and Analysis

The non-ozonated control experiment for Nal^S E. coli consisted of a randomized, two-level factorial design investigating two factors. The variables were growth medium and incubation temperature. The growth media were the standard m-FC agar (APHA, 1985) and a non-selective agar (TLPY) prepared using the same components as m-FC agar, but excluding bile salts no. 3 and rosolic acid. The composition of TLPY was very similar to m-T7 agar (Difco Laboratories, 1984) which was reported to be superior for recovery of injured fæcal coliforms (LeChevallier et al., 1984). All ingredients used in the media were from Difco Laboratories, Inc. except sodium chloride and aniline blue indicator which were from BDH Chemicals Canada Ltd. and Gurr Chemicals, respectively. The two incubation temperatures were 35 and 44.5° C. The treatment combinations for the Nal^S E. coli control experiment are indicated in Table 5.1.

The control experiment for the Nal^R strain was a randomized, two-level factorial design examining three factors, including: growth medium, incubation temperature, and presence or absence of 100 μg of nalidixic acid per mL of growth medium. Media preparation was identical to that for the Nal^S control experiment except that half of each medium contained nalidixic acid. Incubation temperatures were the same as for the Nal^S strain. The details of the treatment combinations are shown in Table 5.2.

Post-ozonation recovery of the Nal^R E. coli strain was investigated using the same factorial design as in the non-ozonated Nal^S control experiment shown in Table 5.1. The growth media did not contain nalidixic acid.

The differences between treatments were analysed by one-way analysis of variance (ANOVA) using the $\log_{10}$ transformation of the observed E. coli densities (Box et al., 1978). Residuals from the ANOVA model were examined to verify the underlying assumptions of ANOVA.

TABLE 5.1 Treatments and results for recovery of the non-ozonated Nal^S E. coli strain and treatments for recovery of the ozonated Nal^R E. coli strain.

Treatment	Growth medium	Incubation temperature, °C	Density of non-ozonated Nal^S strain, log CFU/dL
1	TLPY	35	7.19
2	m-FC	35	7.14
3	TLPY	44.5	7.14
4†	m-FC	44.5	7.14

† APHA (1985) fæcal coliform method

TLPY = trptone, lactose, proteose peptone, yeast extract agar

m-FC = standard fæcal coliform agar

TABLE 5.2 Treatments for recovery of the non-ozonated Nal^R E. coli strain from phosphate buffer.

Treatment	Growth medium	Incubation temperature, °C	Nalidixic acid in medium	Density of Nal^R E. coli, log CFU/dL	
				Run 1	Run 2
1	TLPY	35	No	7.11	7.15
2	m-FC	35	No	7.08	7.04
3	TLPY	44.5	No	7.15	7.15
4†	m-FC	44.5	No	7.08	7.04
5	TLPY	35	Yes	7.11	7.11
6	m-FC	35	Yes	7.11	7.28
7	TLPY	44.5	Yes	7.11	7.08
8	m-FC	44.5	Yes	6.45	6.67

† APHA (1985) fæcal coliform method

TLPY = trptone, lactose, proteose peptone, yeast extract agar

m-FC = standard fæcal coliform agar

5.2.4 Procedure

The Nal^R and Nal^S strains of E. coli were each inoculated from the NA slant into TSB and grown for 18 h at 35° C. A loopful of the 18 h culture was subcultured into TSB and grown for another 18 h at 35° C. A 2.0 mL volume of culture was centrifuged for 10 min at 7,500 x g in a benchtop centrifuge (Model SPX; Sorvall). The resulting pellet was washed once and resuspended in sterile, ozone demand-free 0.05 M phosphate buffer. This was diluted 1:100 in sterile, ozone demand-free phosphate buffer, and 10 mL of this suspension was added to 1000 mL of ozone demand-free phosphate buffer solution. The above procedure produced a mean E. coli density of $10^{7.11}$ CFU/dL.

The suspension of bacteria was constantly stirred using a magnetic stirrer, taking care not to create a turbulent vortex in the flask. A non-ozonated sample was extracted from the Erlenmeyer flask prior to adding the ozone to estimate the initial E. coli density. A 27 mL volume of stock ozone solution was added to 400 mL of 2% potassium iodide solution immediately before adding 9.0 mL of stock ozone solution to the test suspension. Immediately after dosing the test suspension, a second 27 mL portion of stock ozone solution was added to a second 400 mL potassium iodide solution to confirm the dose of ozone applied. The mean ozone dose using this procedure was 0.19 mg/L of solution. The flask was stirred for 60 s at which time the remaining ozone was neutralized with an excess of sterile, 1% sodium thiosulphate solution. The test solution was sampled randomly, in duplicate for each treatment in Tables 5.1 and 5.2. The samples were filtered according to a standard membrane filtration procedure (APHA, 1985) using HAWG47 (Millipore Corp.) membrane filters. The filters were placed on the appropriate growth medium and incubated in accordance with the conditions specified in Tables 5.1 and 5.2, and were counted after 24±2 h of incubation.

5.3 RESULTS AND DISCUSSION

5.3.1 Non-ozonated Control Samples

The biochemical profiles of the Nal^S and Nal^R strains of E. coli determined using the API System 20E strips were identical. The results for the non-ozonated control experiments for the Nal^S and Nal^R strains are tabulated in Tables 5.1 and 5.2, respectively. From the data in Table 5.1, it can be seen that the Nal^S strain was recovered equally well using all treatment combinations. Similarly, from the data in Table 5.2, it is apparent that the Nal^R strain was recovered equally well using the same treatment combinations as the Nal^S strain (treatments 1-4). However, it was found that treatment 8 recovered significantly fewer E. coli than the other seven treatments ($Pr \leq 0.01$). It was postulated that this was the result of an antagonistic interaction between nalidixic acid and rosolic acid at the elevated temperature. This hypothesis was confirmed in a paired trial using nalidixic acid containing m-FC agar with and without rosolic acid. It was found that the m-FC agar with rosolic acid supported the growth of significantly fewer colonies ($Pr \leq 0.05$). Consequently, nalidixic acid was not used in growth media during the ozonation trials.

5.3.2 Post-ozonation Recovery

Seven ozonation trials were performed and Nal^R E. coli was recovered using the four treatments described in Table 5.1. The observed densities for each treatment are tabulated in Table 5.3. ANOVA of the results indicated that there was a marginally significant difference between treatments ($Pr \leq 0.10$) indicating that the differences between treatments should be examined in detail. Examination of residuals from the ANOVA confirmed that the assumptions underlying the procedure were valid.

TABLE 5.3 Post-ozonation Nal^R E. coli densities estimated by the four treatments.

	E. coli density, log CFU/dL			
Treatment	1	2	3	4†
Medium	TLPY	m-FC	TLPY	m-FC
Temperature, °C	35	35	44.5	44.5
Run				
1	1.99	1.86	1.94	1.54
2	1.94	1.79	1.48	1.48
3	—	1.00	1.53	0.95
4	1.93	1.65	1.83	1.42
5	2.18	2.11	2.26	2.08
6	1.82	1.45	1.68	1.20
7	2.18	2.08	2.30	2.04
$\bar{x}$	2.01	1.71	1.86	1.53
SE($\bar{x}$)	0.0586	0.147	0.124	0.156

† APHA (1985) fæcal coliform method

TLPY = trptone, lactose, proteose peptone, yeast extract agar

m-FC = standard fæcal coliform agar

SE($\bar{x}$) = standard error of the mean, $\bar{x}$

Detailed investigation of the differences between individual treatments was done using a graphical technique described by Box et al. (1978). It was found that there was a highly significant ($Pr \leq 0.01$) difference between the treatment pairs 1-4, 1-2, and 3-4. A less significant ($Pr \leq 0.05$) difference was observed between treatment pair 2-4. No significant difference was observed between treatment pairs 1-3 and 2-3.

Treatment 4 is the standard APHA method for enumerating fæcal coliforms. The method uses an agar incorporating bile salts and rosolic acid and an elevated incubation temperature to inhibit non-fæcal organisms. As shown elsewhere (McFeters et al., 1982), bile salts inhibit the recovery of stressed coliform bacteria. The difference between treatment 3 and 4 was probably caused by the bile salts. Also, a marked temperature inhibition was apparent when comparing treatments 2 and 4, where incubation temperature was the only difference between treatments. The m-FC agar incubated at the elevated temperature supported the growth of fewer colonies. In contrast, treatments 1 and 3 used the non-selective medium and had no detectable difference in recovery of bacteria between the reduced and elevated temperature treatments. Both treatments recovered significantly more bacteria than the standard APHA method.

The recoveries of ozone treated E. coli were also investigated using commercially available m-FC agar (Difco Laboratories, Inc.) and an agar, m-T7 (Difco Laboratories, Inc.), which was very similar in composition to TLPY. Five samples were obtained for each growth medium and were incubated at 44.5° C. No significant difference was observed between TLPY and m-T7 ($Pr \leq 0.01$). However, half as many E. coli were recovered using m-FC agar.

Examination of the individual treatment means and their standard errors (Table 5.3) reveals that the most precise estimate of the mean E. coli density was obtained using the non-selective medium at the non-inhibitory temperature (treatment 1) followed by the same medium at the elevated temperature (treatment 3). The least precise estimate of the mean density was achieved using the standard APHA method (treatment 4). These observations were consistent with Haas and Heller (1986) who suggested that recovery of stressed coliforms using inhibitory media may contribute to variances greater than those expected from a Poisson distribution.

The results reported here indicate that the standard MF method for enumerating fæcal coliform bacteria in ozone disinfected water may underestimate the bacterial density. Faecal coliform bacteria are the most commonly used indicator organisms for measuring the performance of wastewater disinfection processes, for both research and regulatory purposes. Therefore, it is necessary that the significance of underestimating fæcal coliform indicator bacteria in disinfected waters be understood before using the standard MF procedure.

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6. DOSE-RESPONSE OF E. COLI IN OZONE DEMAND-FREE PHOSPHATE BUFFER

6.1 INTRODUCTION

Ozone dose-response in demand-free water has generally been ancillary to kinetic studies investigating the response of bacteria to selected residual concentrations of ozone over a range of contact times. A frequently cited example of this type of study is that by Katzenelson et al. (1974). Kinetic studies of this type have direct significance in water disinfection but they are not completely relevant in wastewater disinfection. Smith (1967) examined the dose-response of E. coli and compared his results with those from several other studies (Fetner and Ingols, 1956; Holluta and Unger, 1954; Wuhrmann and Meyrath, 1955). He found that there were some important differences between the results which he attributed to the methodology of the different investigators such as means of introducing ozone into solution and insufficient care in the preparation of bacterial cultures, ozone demand-free glassware and and demand-free water.

Extrapolation of disinfection data from potable water studies to wastewater disinfection studies must be considered with care. There are several differences between wastewater disinfection and potable water disinfection which can cause difficulties in comparing responses in "clean" water systems with those in wastewater. The complete elimination of indicator bacteria is not usually needed in wastewater disinfection as it is in potable water disinfection. A common requirement in wastewater disinfection is to reduce indicator bacteria to levels which minimize the risk of illness resulting from recreational activities in surface waters receiving wastewater discharges. A second major difference between water and wastewater disinfection is the method of controlling the disinfection process. Control of ozone disinfection in water is commonly managed by maintenance of a residual for a minimum contact time (Légeron, 1984). In wastewater, this is not practical since there are many competing oxidation reactions other than disinfection, which tend to cause rapid reduction of the ozone residual (Rice et al., 1982). Also, a reliable device for continuously monitoring ozone residual in wastewater has not been reported (United States EPA, 1986). A more commonly used approach in wastewater disinfection systems has been process control through off-gas monitoring (Venosa and Meckes, 1983). The difference between the mass of applied ozone and the mass of ozone in the off-gas is known as the transferred dose and can be correlated to disinfection efficiency in the absence of residual ozone (Venosa et al., 1985). Therefore, the transferred ozone dose required to achieve the desired response in the indicator bacteria population is a more important design parameter than the maintenance of an ozone residual as in water treatment.

In view of the discrepancies in the published dose-responses and considering the importance of the dose-response relationship to wastewater disinfection, it was concluded that there was a need for a detailed investigation into the ozone dose-response relationship using a known strain of fæcal coliform bacteria. The purpose of this study was to provide a reference dose-response relationship for ozone disinfection of E. coli in ozone demand-free water. The experimental protocol was designed to facilitate comparison of the data from this study with data presented in Chapter 7, where these experiments were repeated using secondary effluent from an activated sludge wastewater treatment plant.

6.2 MATERIALS AND METHODS

6.2.1 Ozone Apparatus

Ozone was produced from extra dry oxygen using the same apparatus and conditions as in Chapter 5. The concentration of the stock ozone solution was determined as described in Chapter 5 using the method of Birdsall et al. (1952). The concentration of ozone in the stock solution ranged from 20 to 23 mg/L at 20° C. The colorimetric indigo procedure described by Bader and Hoigné (1981, 1982) was also used to determine the concentration of the stock ozone solution using potassium indigo trisulphonate (Riedel de Haën AG). This reagent had a reported molar absorption coefficient of 0.42 $\frac{L}{mg \cdot cm}$ at 600 nm (Bader and Hoigné, 1981, 1982). A spectrophotometer (Spectronic 21 DV; Bausch and Lomb) with a maximum light path of 2.5 cm was used to measure changes in absorbance. The indigo method was used to measure residual ozone in the disinfection trials. Ozone demand-free glassware and phosphate buffered water were prepared as described in Chapter 5.

6.2.2 Bacteriological Methods

E. coli strain 11775 from the American Type Culture Collection (ATCC®) was used to obtain a nalidixic acid-resistant mutant strain, as described in Chapter 5. This was done to provide a marker organism which could be readily distinguished in ozone disinfection studies where mixed species of microorganisms were present.

The seed culture was prepared as described in Chapter 5. The mean E. coli density in the seeded 0.05 M phosphate buffer (pH 6.9) was $10^{7.22}$ CFU/dL. The standard deviation of the 57 trials was 0.0851 log units. Controls were periodically performed with E. coli seeded in the phosphate buffer to determine if undue die-off occurred in the absence of ozone. No significant change was observed in the E. coli density for time periods of up to 40 min.

The E. coli were enumerated by membrane filtration using Millipore HAWG47 membrane filters (APHA, 1985). Dilution series were performed using sterile, 0.1% peptone diluent which has been shown to enhance the recovery of stressed coliforms at room temperature (McFeters et al., 1982). The filters were placed on the non-selective growth medium, TLPY, evaluated in Chapter 5 and were incubated at 44.5 ± 0.2° C for 20 ± 2 h.

6.2.3 Procedure

The experiments were performed randomly using five dose levels examined at contact times of 30, 60, and 120 s. The mean applied ozone dose levels were 810, 200, 45, 11, and 4.4 μg/L. Each 1000 mL volume of ozone demand-free phosphate buffer comprised a single experimental trial.

The suspension of bacteria was constantly stirred using a magnetic stirrer, taking care not to create a turbulent vortex in the flask. No baffles were used. Prior to ozonation, duplicate, 1.0 mL samples were taken from the Erlenmeyer flask to estimate the initial E. coli density for each trial. The stock ozone solution concentration was measured by the potassium iodide method immediately before and after the addition of a known volume of stock solution to the bacterial suspension. The mean concentration was used to calculate the applied dose. The trial was redone if the repeat analyses were different by more than 5%. The applied mass of ozone per litre was calculated from:

$$V_S \cdot C_R \qquad [6.1]$$

where V_S is the volume of stock ozone solution and C_R was the concentration of the stock solution. Since there was no off-gas to account for, the applied dose in these trials was analogous to transferred dose in systems through which the gas was continuously bubbled.

The ozone treated E. coli suspension was continuously stirred for the desired contact time and any remaining ozone was neutralized with an excess of sterile, 0.1% sodium thiosulphate solution. The test solution was sampled randomly, in triplicate, to confirm that the underlying statistical assumptions for membrane filtration were satisfied according to the method described by Haas and Heller (1986). As experience was gained in bacteriological procedures, replication was reduced to duplicates. If the deviation from the Poisson distribution was significant at the 1% level, the trial was repeated.

Ozone residual was not determined for each trial since the indigo method for ozone analysis was not introduced until near the end of the experimental program. However, owing to the uniformity of the experimental conditions over the study period, it was believed that performing additional randomized trials would provide reliable information on the typical mass of ozone utilized at each dose level and contact time. During these trials, the indigo method was also used to measure the concentration of the stock ozone solution. There was no practical difference between the potassium iodide and the indigo methods when determining the stock ozone solution concentration. However the potassium iodide method gave results which were approximately 5% higher than the indigo method. The difference between the applied ozone and residual ozone was the utilized ozone.

6.3 RESULTS

Raw data collected from these experiments are tabulated in Appendix F. There were 28 trials conducted at 60 s contact time, 14 trials at 30 s contact time and 15 trials at 120 s contact times for a total of 57 independent observations. The observed response ($\log\frac{N}{N_0}$) of E. coli at each dose level and contact time is summarized for the 57 trials in Table 6.1. The 95% confidence limits for the mean response of E. coli at each applied ozone dose and contact time are plotted in Figure 6.1. The confidence limits for the response data were obtained by confirming that the variance of the response distribution at each dose level was approximately constant using a graphical procedure described by Box et al. (1978). A pooled estimate of the experimental error was made using

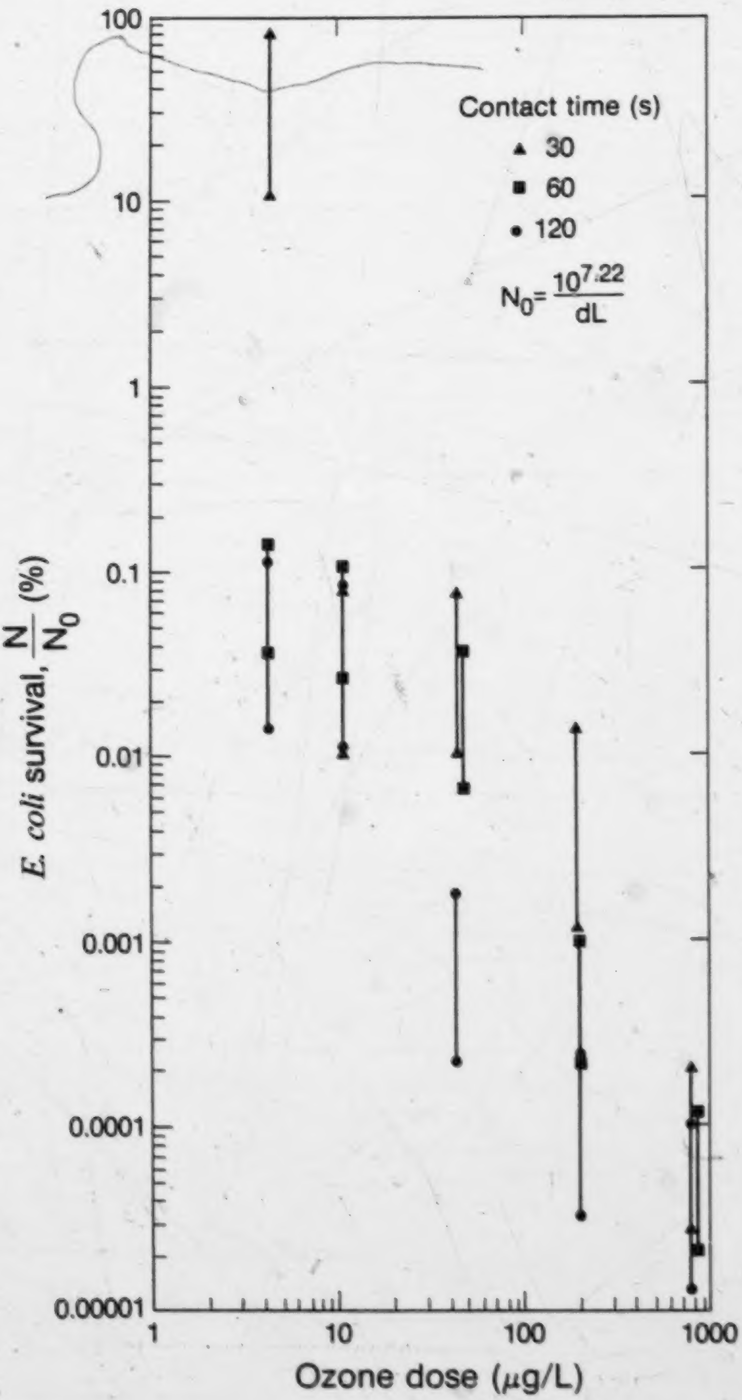
TABLE 6.1 Summary of dose-response data collected for ozone disinfection of *E. coli* in ozone demand-free phosphate buffer for different contact times and dose levels.

Contact time, s	Dose level	Mean ozone dose, µg/L	Mean utilized ozone, µg/L	Mean *E. coli* survival, $\log N/N_0$	Number of trials	95% confidence limits for $\log N/N_0$‡	
						Lower	Upper
30	1	790	160	-6.13	3	-6.57	-5.69
	2	190	29	-4.39	2	-4.93	-3.85
	3	45	11	-3.56	3	-4.00	-3.12
	4	11	5	-3.54	3	-3.98	-3.10
	5	4.5	4.5•	-0.527	3	-0.970	-0.0840
60	1	840	180	-6.30	4	-6.68	-5.92
	2	200	36	-5.30	7	-5.59	-5.01
	3	47	9	-3.81	4	-4.19	-3.43
	4	11	6	-3.28	6	-3.59	-2.97
	5	4.4	4.4•	-3.14	7	-3.43	-2.85
120	1	790	220	-6.46	3	-6.90	-6.02
	2	200	53	-6.06	3	-6.50	-5.62
	3	44	13	-5.18	3	-5.62	-4.74
	4	11	6	-3.53	3	-3.97	-3.09
	5	4.4	4.4•	-3.41	3	-3.85	-2.97

‡ Error variance was 0.144 with 42 degrees of freedom

• No detectable residual ozone

FIGURE 6.1 95% confidence limits for the mean response of E. coli to ozone in ozone demand-free phosphate buffer.



the 57 observations (Davies and Goldsmith, 1984). The error variance calculated using this method was 0.144 with 42 degrees of freedom.

The formation of ozone residual as a function of applied dose is presented in Figure 6.2. Each observation was obtained from the mean of 3 trials. Ozone residual developed as a linear function of applied ozone dose from 45 to 810 μg/L with approximately 70 to 80% of applied ozone appearing as residual.

The kinetics of ozone decomposition in water may vary. However, Masschelein (1982) noted that first-order kinetics generally described the decomposition of ozone. Figure 6.3 is a plot of the logarithm of ozone concentration in phosphate buffer as a function of the contact times used in this study. If the decay of ozone is first-order, the slope of a straight line on this plot would be an estimate of the rate constant (Grady and Lim, 1980). The data shown in Figure 6.3 suggest that ozone decomposition is approximately first-order for the three largest doses. The lowest dose level demonstrated some deviation from a straight line. The data were also plotted assuming second-order kinetics but were found to be unsatisfactory. The approximate first-order rate constant was estimated by the method of least-squares at each dose level. The mean value of the rate constant was -7.6×10^{-4}/s. Therefore, the calculated half-life of ozone, assuming complete mixing, was 1300 s.

The 95% confidence limits of the E. coli response in terms of the utilized ozone dose are plotted in Figure 6.4. Figure 6.5 is a plot of the mean response at each utilized ozone dose which is compared with the data of Dahi (1977) who performed a similar experiment without replication.

The surviving fraction of E. coli were plotted on in Figure 6.6 as a function of contact time. If the disinfection reaction was first-order, the data at each dose level would plot as a straight line with a slope equal to the reaction rate constant and an ordinate axis intercept at 100% survival (Grady and Lim,

FIGURE 6.2 The formation of ozone residual as a function of ozone dose and contact time in the presence of $10^{7.22}$ E. coli per dL in ozone demand-free phosphate buffer.

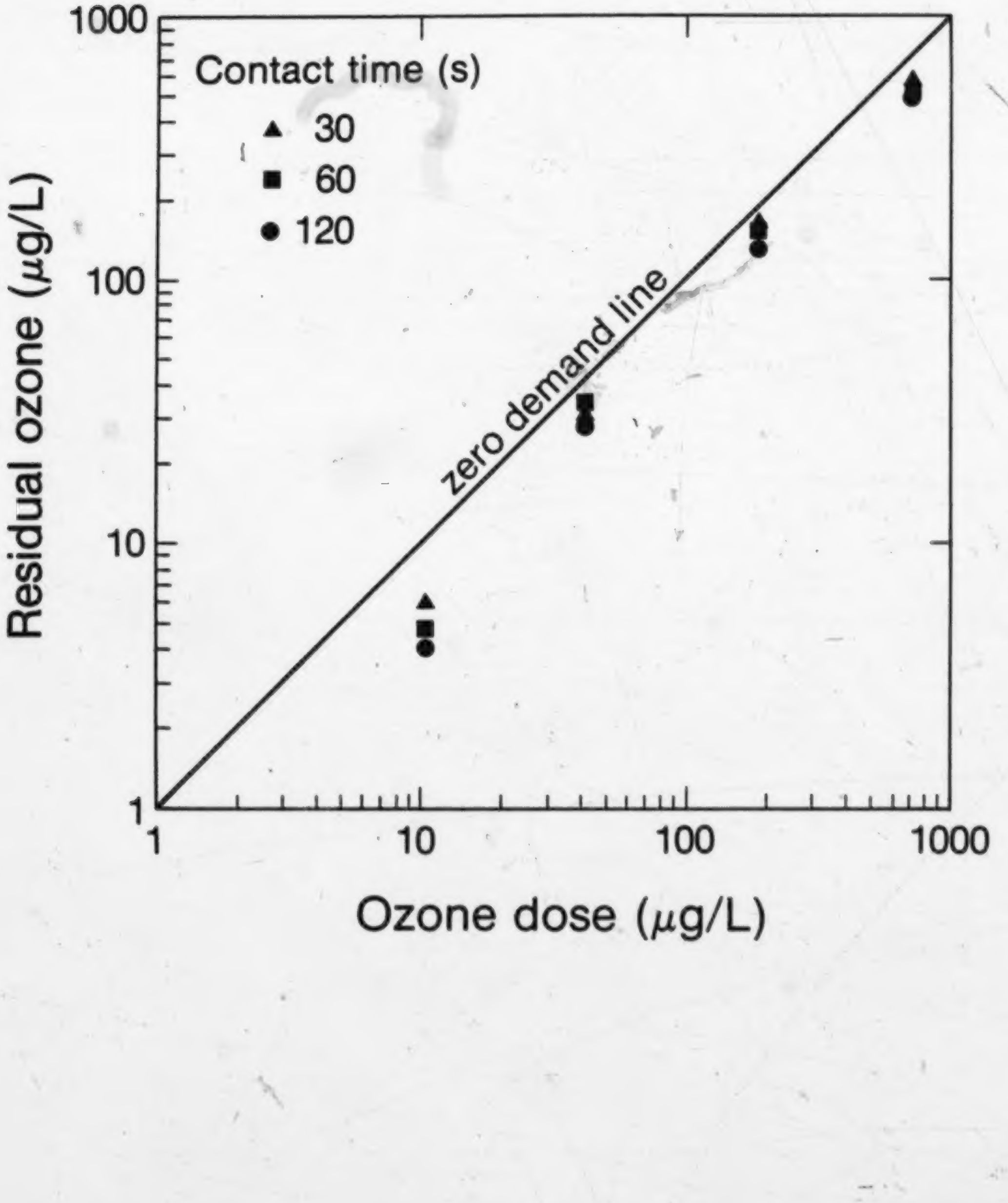


FIGURE 6.3 Decay of ozone residual in 0.05 M phosphate buffered, ozone demand-free water in the presence of $10^{7.22}$ E. coli per dL.

Ozone dose
- ● 810 μg/L
- ▲ 200 μg/L
- ○ 44 μg/L
- □ 11 μg/L

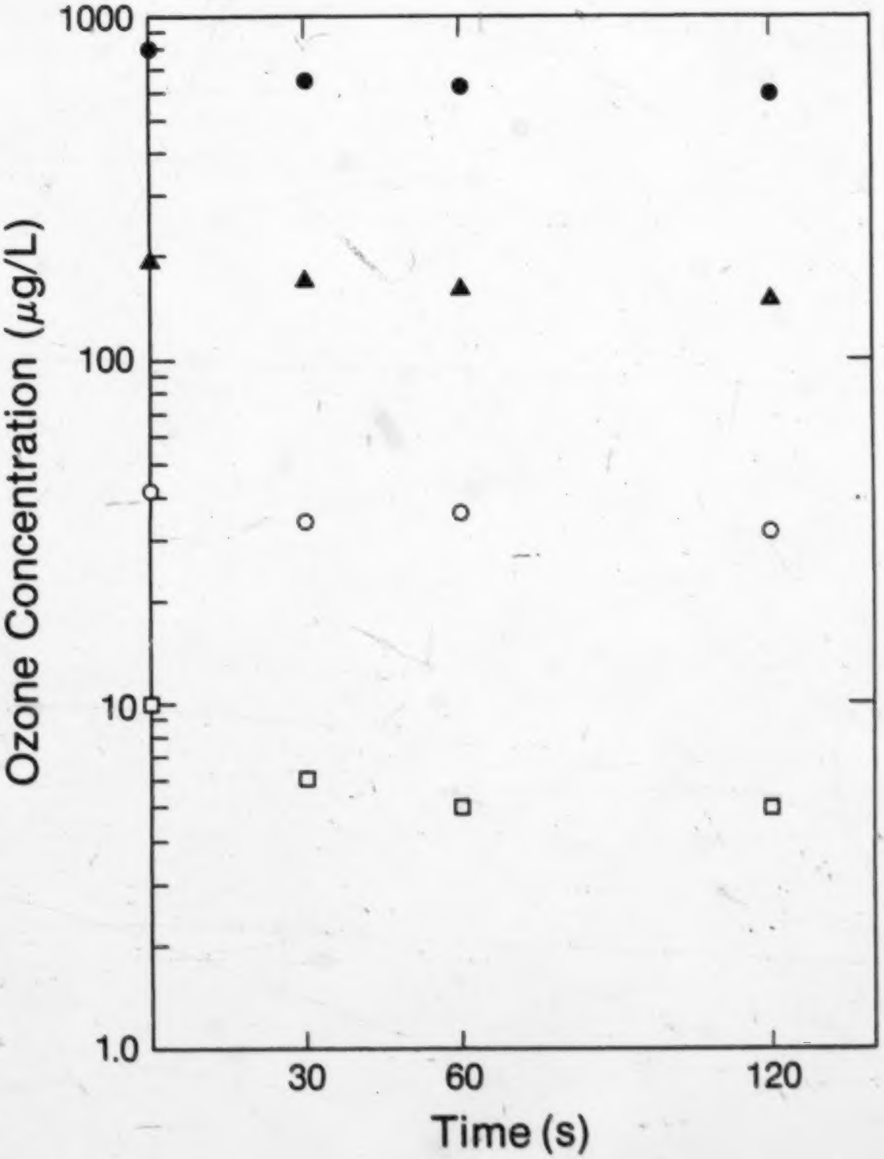


FIGURE 6.4 95% confidence limits for the mean response of E. coli to estimated utilized ozone in ozone demand-free phosphate buffer.

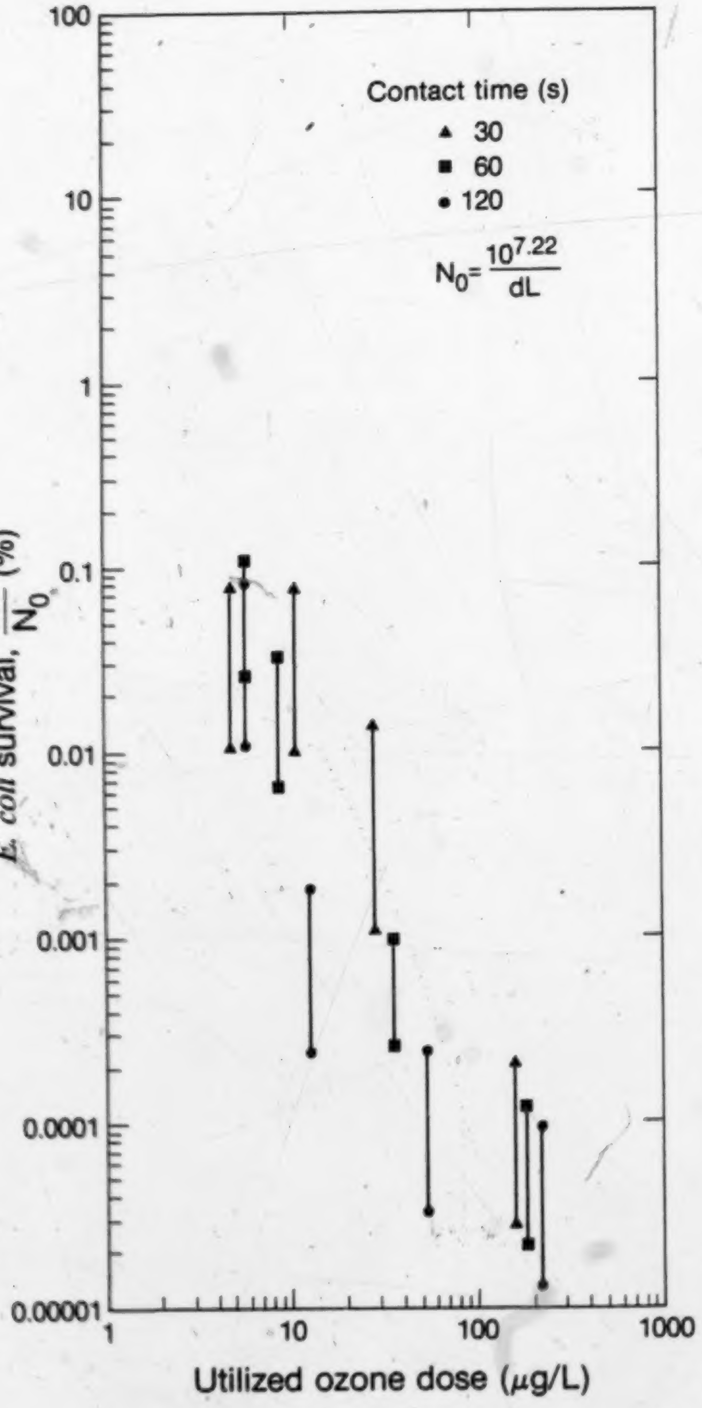


FIGURE 6.5 The mean response of E. coli at each utilized ozone dose compared with a similar, unreplicated study from the literature.

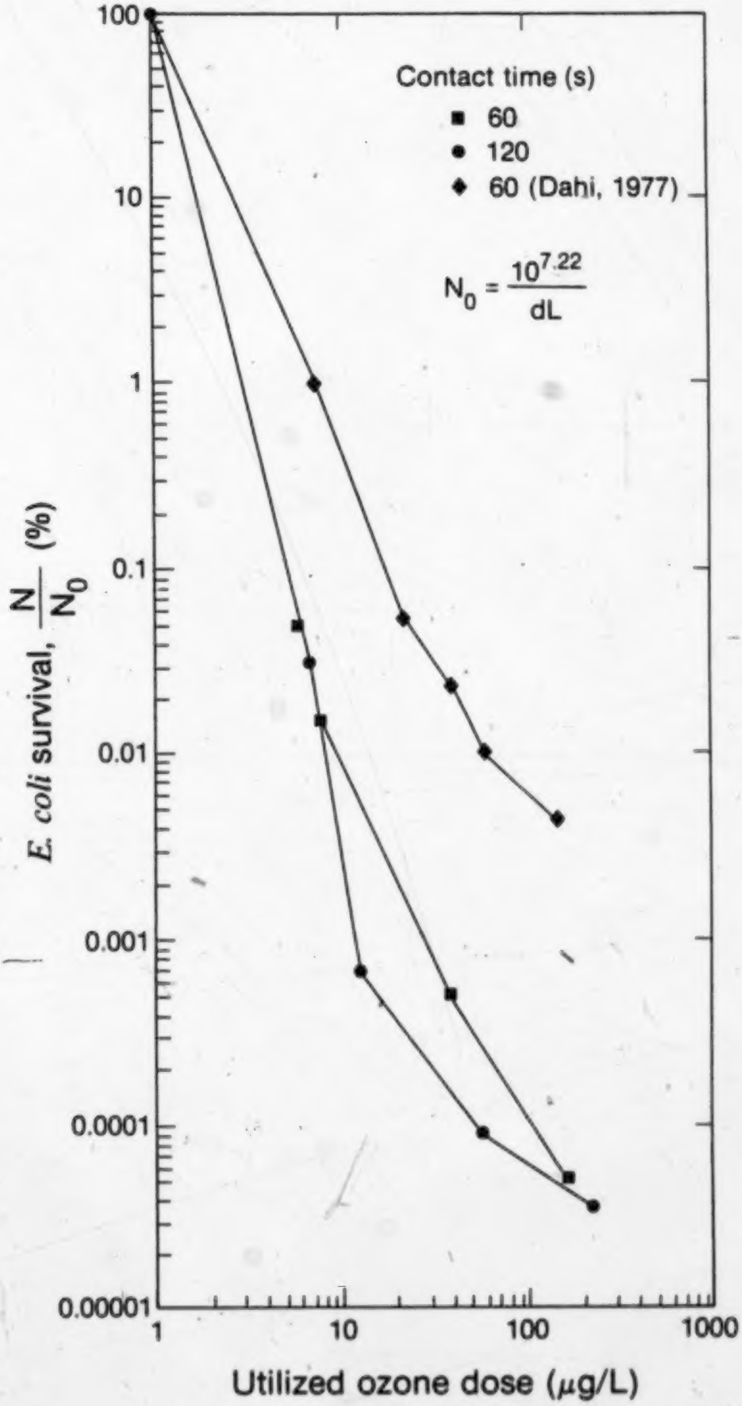
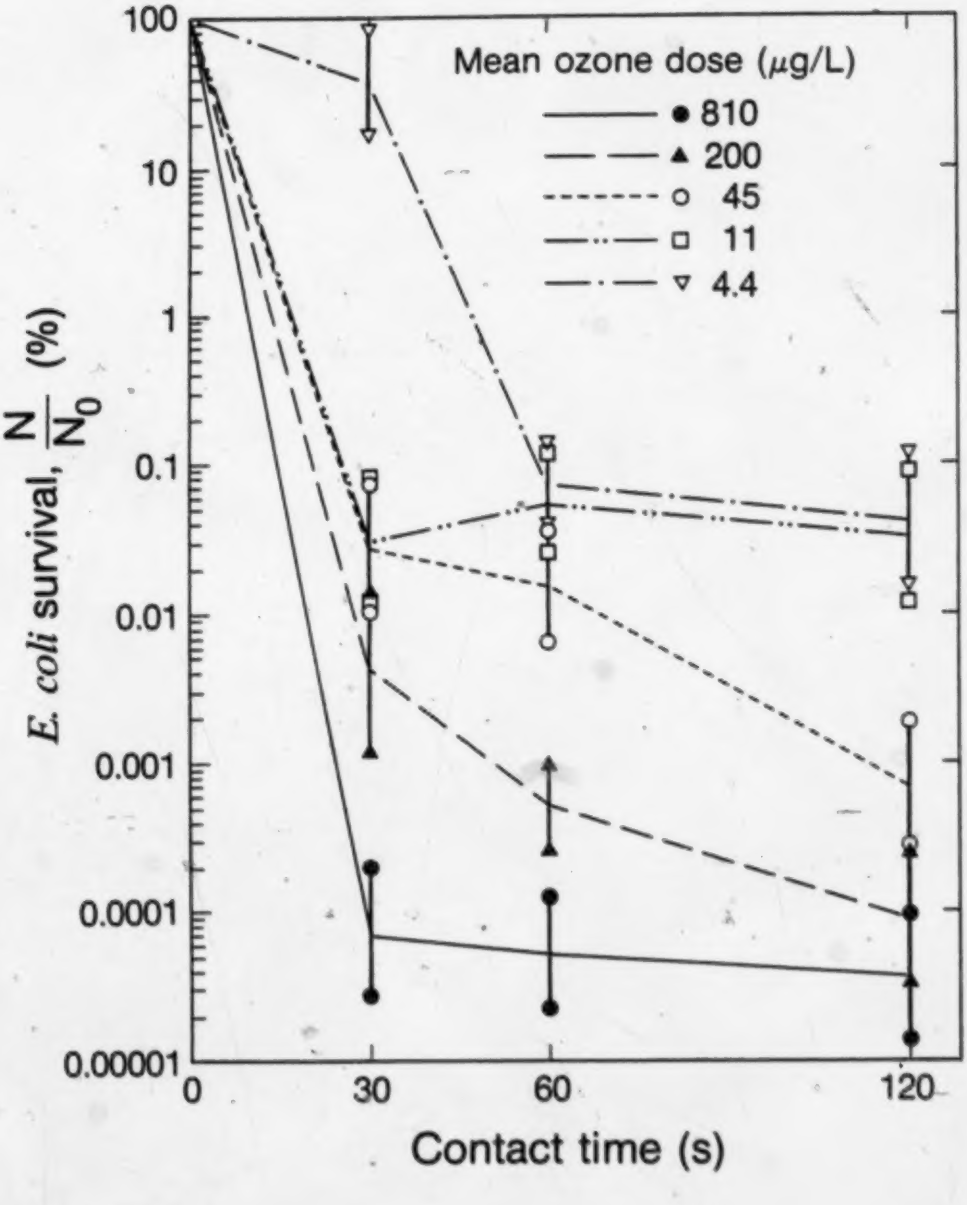


FIGURE 6.6 95% confidence limits of the mean response of E. coli at various ozone dose levels as a function of contact time.



1980). It is evident from Figure 6.6, that the reaction is not of first-order. The disinfection rate in the first stage was fast and was complete within 30 s except in the case of the lowest dose where it was complete within 60 s. The contact times examined in this study did not delineate the complete experimental region for the first stage. Regression analysis of the second stage at each dose level did not reveal a significant time-dependent reduction of E. coli except for the region between applied ozone doses of 45 and 200 μg/L where a significant decline in viable E. coli was associated with contact time.

Two E. coli colonies which survived an ozone dose of 810 μg/L for 120 s were picked from the TLPY and were prepared for seeding as described earlier. Subsequent ozone treatment in parallel with a control using the non-ozonated E. coli seed did not reveal any difference in resistance to ozone, suggesting that survival at the higher applied doses was not the result of a genetically transmitted resistance to ozone.

In an ozone demand-free system, the only source of ozone demand is the seeded organisms. Scott and Lesher (1963) reported only slight immediate lysis of cells exposed to ozone with less than 50% of the cells being affected. Since they had approximately 10^8 cells per mL in their test solution, 50% represented a large number of lysed cells. Therefore, even slight cell lysis may generate a detectable ozone demand in an otherwise demand-free water. To estimate the ozone demand potential of cell lysis, a sonic cell destructor (Model S-75, Branson Instruments Inc.) was used to destroy 10^6 E. coli cells in 8 mL of ozone demand-free phosphate buffer. The sonic energy was 4.0 grams and was applied for 10 min in a temperature controlled rosette. The solution of treated cells was added to a 250 mL demand-free Erlenmeyer flask containing 100 mL of demand-free phosphate buffer. Stock ozone solution containing 600 μg of ozone was added to the solution and to a blank. After 60 s, the residual ozone was determined. This experiment was duplicated and it was found that

60 μg of ozone were consumed by the solution.

6.4 DISCUSSION

6.4.1 Ozone Dose-response

Caverson et. al. (1986) summarized some of the dose-response relationships reported in the literature for ozone disinfection of wastewater. Typically, the models were empirically obtained from regression analysis of disinfection data collected from continuous pilot-plant operations operated under a variety of conditions of ozone dose, contact time, and wastewater strength. None of the models discussed included contact time as a parameter. The method of least-squares was used to estimate the parameters, β_0 and β_1, for model [4.2] from all of the data shown in Figure 6.1. The resulting model was:

$$\log\frac{N}{N_0} = -1.61 - 1.61 \log C_A \qquad [6.2]$$

where N is the concentration of surviving bacteria, N_0 is the initial concentration of bacteria, and C_A is the applied ozone dose. The R^2 value was 0.76. Analysis of variance of the regression using the methods described by Draper and Smith (1981) revealed that there was significant lack-of-fit ($Pr \leq 0.01$). Details of the formal test for lack-of-fit are provided in Appendix K.

Insertion of contact time into the model resulted in the following dose-response model:

$$\log\frac{N}{N_0} = -0.730 - 1.60 \log C_A - 0.0132\ t \qquad [6.3]$$

where t is the contact time. This model explained more of the variation in the data than the previous one ($R^2 = 0.84$). However, analysis of variance revealed that there was significant lack-of-fit ($Pr \leq 0.01$).

The calculated applied dose intercepts for 100% survival from models [6.2] and [6.3] were 0.10 and 0.35 μg/L, respectively. Therefore, if lack-of-fit was not checked, models [6.2] and [6.3] would appear to be good predictors of disinfection performance. However, there is lack-of-fit in both models and they are inadequate predictors of performance.

The reason for lack-of-fit is apparent from inspection of Figure 6.1. There is non-linearity between applied dose and the responses at ozone doses less than 45 μg/L. Regression analysis using the data from 45 to 810 μg/L results in a model which has no lack-of-fit:

$$\log\frac{N}{N_0} = -0.427 - 1.72 \log C_A - 0.0130 \cdot t \qquad [6.4]$$

The R^2 value was 0.86 and the 100% survival applied dose intercept was 0.56 μg/L. It appears that it is the dose region from 4.4 to 45 μg/L that produced the lack-of-fit. These examples illustrate that empirical models may only be adequate over limited ranges of the experimental region studied and that caution should be exercised in using empirical models beyond the region for which they were developed.

6.4.2 Dose-response in Terms of Chemical Kinetics

The appearance of Figures 6.1, 6.5, and 6.6 could be explained in terms of chemical kinetic theory by assuming that disinfection is described by the following general reaction:

$$aA + bB \rightarrow \text{products} \qquad [6.5]$$

The approximate reaction rate for the above reaction is (Grady and Lim, 1980):

$$r_B = \frac{dC_B}{dt} = k\ C_A^{\,a}\ C_B^{\,b} \qquad [6.6]$$

Consider that compound A is ozone and and that compound B is a bacterium. The rate of disappearance of either component is the reaction rate, r, which is a function of the concentration of both compounds. The maximum rate of reaction occurs when both components are at their initial concentrations. However, as either A or B approach a concentration of zero, the overall reaction rate approaches zero as well. Masschelein (1982) and Hoigné (1982) have suggested that the overall rate constant, k, for the disinfection reaction is large. Therefore, in the presence of a sufficient concentration of bacteria, the reaction will be rate-limited by the supply of ozone. If ozone is supplied in excess, the disinfection reaction progresses and the concentration of surviving bacteria becomes rate-limiting. Between these two extremes, the reaction rate is dependent upon the concentrations of surviving bacteria and ozone. This concept can be used for interpretation of the results.

As postulated above, if the reaction rate was slow as a result of a low concentration of ozone, the change in concentration of surviving bacteria may be detectable over the contact times used in this study. This was the case at the 4.4 μg/L dose level where the concentration of surviving bacteria declined between 30 and 60 s (Figure 6.6). After 60 s of contact, no further reduction in E. coli was observed suggesting that the disinfection reaction rate had declined to approximately zero. At the other extreme condition (810 μg/L), where an excess of ozone was available and the concentration of surviving bacteria was low, there was no appreciable change in the concentration of surviving bacteria over the contact times investigated (Figure 6.6). This observation is consistent with a disinfection reaction rate approaching zero.

Between the two extreme conditions of ozone concentration and surviving bacteria, it is expected that a range of reaction rates, r, will be observed depending on the relative concentrations of the reacting components. The results for the intermediate doses of ozone, 11, 45, and 200 μg/L, support this prediction. There was no detectable difference in surviving E. coli between any of the contact times used at 11 μg/L of applied ozone. This observation may have been the result of insufficient contact time to determine an effect if a very slow reaction rate had resulted from low concentrations of ozone and

E. coli. At 45 μg/L of applied ozone, a detectable disinfection reaction rate was observed after 60 s of contact. This trend was also observed at the 200 μg/L dose level. Neither of these dose levels had sufficiently long contact times to determine when the reactions were complete.

The disinfection process can be visualized as a stochastic process where billions of ozone molecules are uniformly distributed in an aqueous solution. Scott and Lesher (1963) estimated that 4 x 10^7 molecules of ozone are required to inactivate a single E. coli cell when 1% of 10^9 cells per mL survived ozonation. Since the reaction rate is a function of the frequency of the collisions between the bacterium and the ozone molecules and if ozone is consumed during the reaction, the utilized ozone should be correlated with the number of bacteria removed.

E. coli survival as a function of utilized ozone was presented in Figure 6.4 and 6.5. Only 4 to 5 μg/L of ozone were actually consumed in removing the first 3.5 log units of E. coli. Assuming 4.5 μg of ozone reacted with the bacteria which were removed, this corresponds to 3 x 10^8 molecules of ozone per bacterium. This is within an order of magnitude of the estimate by Scott and Lesher (1963). However, the evidence presented in Figures 6.1, 6.4, and 6.6 indicates that a change occurs in the dose-response relationship after 4.5 μg/L of ozone has been added to the system. Also, a 7 log unit reduction in E. coli required 45 times more ozone than predicted by the apparent stoichiometry of 3 x 10^8 molecules of ozone per bacterium. This creates a dose-response relationship which has a "tail", as increasingly larger doses of ozone remove increasingly fewer bacteria.

As discussed in Chapter 1, one possible explanation of this phenomenon is that the population of bacteria contains individuals more resistant to the effects of the disinfectant. It was found in this study, that viable bacteria at the highest dose level did not have a resistance to ozone which was passed on to daughter cells. However, as was also discussed in Chapter 1, the age distribution of the organisms in the culture may cause the same effect.

Another possible explanation is that cellular debris from the disabled organisms shields the surviving *E. coli* from the effects of ozone. Evidence for this possibility exists in Figure 6.1 where equivalent reductions of *E. coli* were achieved by 200 and 810 μg/L of applied ozone at 120 s contact time. Between these two dose levels, ozone utilization increased 400% but no detectable decrease in bacteria was observed. The shielding phenomenon was considered by Katzenelson *et al.* (1974) who concluded that the lack of change in response after 60 s of contact in the presence of greater than 70 μg/L of ozone was caused by clumped bacteria. Dahi (1976, 1977) investigated the effects of ultrasound on the inactivation of *E. coli* using ozone in a demand-free phosphate buffer. He observed that in solutions which were not sonically treated before ozonation, the tailing effect occurred. However, after 2 to 4 min of sonication before ozonation, the tailing effect was not apparent. His experiments did not determine if pretreatment with sonic energy predisposed *E. coli* to the effects of ozone in addition to breaking clumps.

Hoigné (1982) demonstrated by the use of reaction rate data that the shielding effect can be obtained when competing reactions for ozone have a faster reaction rate than the disinfection reaction. Consequently, as the ozone dose increases and surviving bacteria concentration decreases, cell lysis may occur and since the disinfection reaction rate may be decreasing, competing reactions for ozone may be successful in using ozone. It was found in this study that 10^6 *E. coli* cells consumed 60 μg of ozone. Therefore, an appreciable ozone demand may occur as cells lyse, thereby shielding remaining viable organisms.

6.4.3 Comparison with Other Studies

The results of five published studies which examined ozone disinfection of E. coli in demand-free water have been plotted in Figure 6.7 with the mean survival of E. coli from this study. Table 6.2 summarizes the experimental conditions used in these experiments. The investigators took particular care in the preparation of ozone demand-free water and glassware, and in the preparation of bacterial cultures.

The data reported by Dahi (1976, 1977) were similar in appearance to the data found in this study. At the lower ozone concentrations, the reductions in E. coli were comparable. He measured ozone residual and surviving bacteria simultaneously. An ozone residual of approximately 45 μg/L resulted in greater than 99.9% reduction in viable E. coli. From Figure 6.2, an ozone residual of 45 μg/L would result from an applied dose of about 55 μg/L. The utilized ozone would then be approximately 10 μg/L which is consistent with the results from this study summarized in Figure 6.4. His data were plotted in terms of utilized ozone in Figure 6.5. The resulting plot is similar to the plot obtained from this study (Figure 6.5), except his ozone doses were somewhat higher. This may have been a function of the difference in experimental protocol such as the method for ozone residual measurement or the strain of bacteria used. .

Katzenelson et al. (1974) reported maintaining a constant ozone residual for up to 15 min without adding ozone to the batch reactor. Dahi (1976) could not duplicate those conditions. Also, Katzenelson et al. (1974) did not report results where the residual dropped by more than 20% of the initial ozone concentration. Therefore, if the ozone residual for the reported dose-response conditions, 40 to 70 μg/L, was less than or equal to 20% of the initial concentration, this would correspond to utilized ozone of less than or equal to 8 to 14 μg/L. They reported 99.9 to 99.99% removal under these conditions which is similar to the data shown in Figure 6.4 where 3 to 4 log unit reductions

FIGURE 6.7 The mean response of E. coli to ozone from this study compared with selected published data from disinfection studies of E. coli in ozone demand-free water.

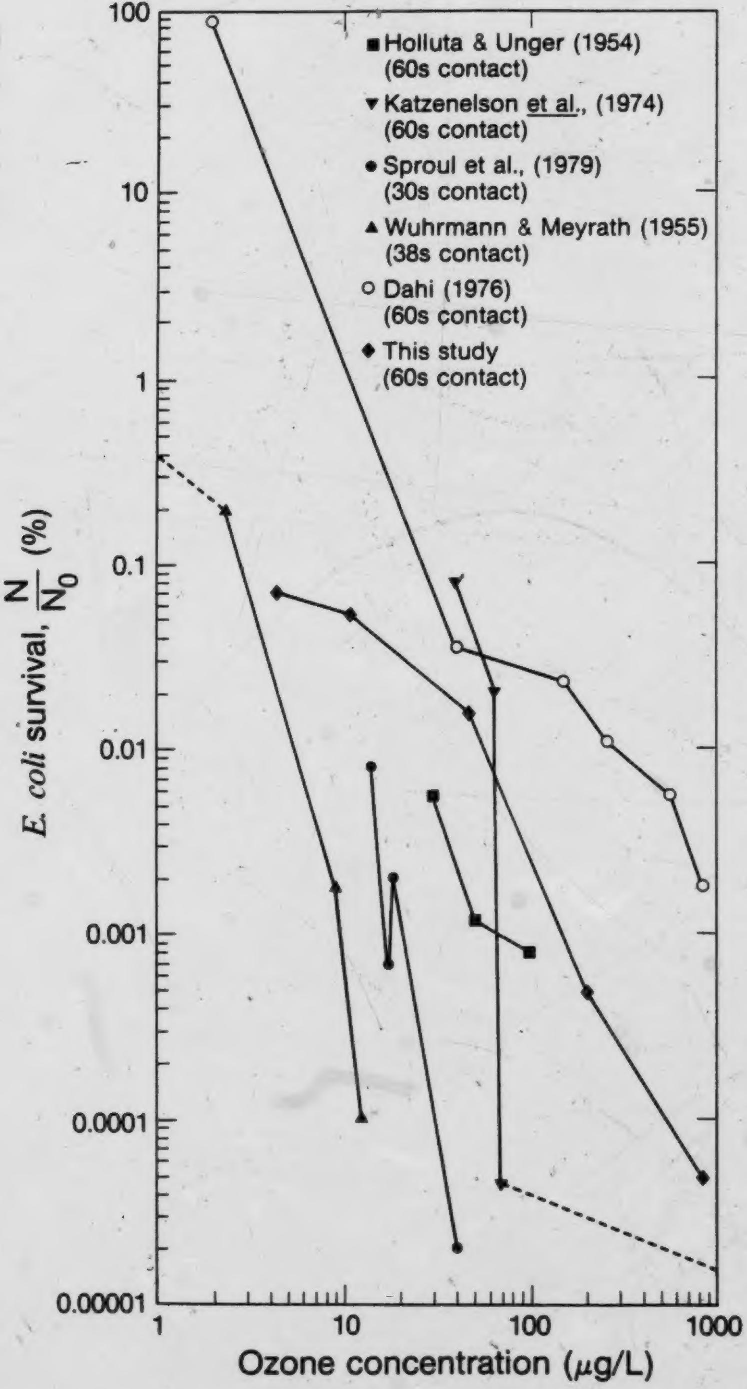


TABLE 6.2 Summary of experimental conditions used in some ozone disinfection studies reported in the literature for E. coli in ozone demand-free water.

Concentration range, μg O_3/L	Contact time, s	Initial cell density, CFU/dL	E. coli analysis method	Type of water	pH	Temperature, °C	Number of replicates	Ozone analysis method	Reference
2-810	0-600	$10^{7.75}$	PPTG	PB	7.2	21	1	Schecter (1973)	Dahi (1976)
30-100	60, 300	$10^{5.38}$	N/R	distilled water	5.6	20	1	KI	Holluta & Unger (1954)
20-1550	10-120	$10^{7.70}$	PPNA	PB	7.0	1	3	Schecter (1973)	Katzenelson et al. (1974)
14-260	10-40	10^{8-10}	PPNA and MPN	PBS	7.0	20	1	Schecter (1973)	Sproul et al. (1979)
0.6-125	10-140	$10^{7.48}$	N/R	CO_2-$NaHCO_3$ buffer	7.0	12	3	KI	Wuhrmann & Meyrath (1955)
4.4-810	30-120	$10^{7.22}$	MF on TLPY	PB	6.9	20	2-7	KI and indigo	This study

KI - potassium iodide
MPN - most probable number
PB - phosphate buffer
PPNA - pour plate in nutrient agar

TLPY - tryptose, lactose, proteose peptone, yeast extract agar

MF - membrane filtration
N/R - not reported
PBS - phosphate buffered saline
PPTG - pour plate in glucose extract agar

were typical for this amount of utilized ozone.

Holluta and Unger (1954) explored a narrow range of ozone concentrations which is reflected by the narrow response region they observed. They reported 99.99% reduction of E. coli with an ozone residual of 30 μg/L. This ozone residual would result from an applied ozone dose of approximately 45 μg/L (Figure 6.2). As shown in Figure 6.1, an applied dose of 45 μg/L could achieve similar reductions to those reported by Holluta and Unger (1954).

The results of Wuhrmann and Meyrath (1955) were similar to those reported here for the lower applied doses. However, at the higher doses, considerably less ozone was required in their study. Sproul et al. (1979) reported a 99.99% reduction of viable E. coli using an applied dose of 14 μg/L which was similar to the data reported here.

In general, the literature supported the observation that only a few micrograms of ozone per litre were sufficient to reduce E. coli by greater than 99.9% in less than 60 s in ozone demand-free water. The differences in results for higher concentrations of ozone are likely artifacts resulting from differences in the physiological state of the test bacteria, the type of experimental protocol and the means of describing disinfectant concentration; that is, applied, utilized, or residual ozone. There were also several potential sources of systematic error. For example, in the methods for:

1. applying ozone to the system;
2. enumerating viable bacteria; and
3. analysis of ozone in aqueous solutions.

Methods of applying ozone to the test water varied from study to study. Some prepared a batch of ozone demand-free test water containing a known ozone residual to which was added the prepared bacterial seed (Dahi, 1976; Holluta and Unger, 1954; Katzenelson et al., 1974). Whereas others added a known volume of concentrated stock ozone solution to a batch of demand-free water containing a bacterial seed (Smith, 1967). In some studies using semi-batch or continuous flow systems, ozone was bubbled through demand-free water containing the seeded bacteria (Sproul et al., 1979; Wuhrmann and Meyrath, 1955).

Since a commonly used criterion for potable water disinfection using ozone is the maintenance of an ozone residual for a specified contact time, the survival of E. coli in the presence of an ozone residual is an important consideration. However, since ozone is consumed relatively quickly in wastewater, maintenance of an ozone residual is economically unattractive. Consequently, protocols where ozone was bubbled through the water accompanied by an accurate ozone mass balance of the system may better approximate the conditions of wastewater disinfection. Adding known volumes of a stock ozone solution is similar to bubbling ozone through water except mass transfer considerations are not relevant and imprecise measurements of ozone in the gas phase are eliminated.

The methods for estimating the number of viable E. coli in aqueous solutions include the spread or pour-plate procedure, the most probable number (MPN), and membrane filtration (MF). Standard Methods (APHA, 1985) documents the relative merits of each procedure. In clean water, the membrane filtration procedure is the most precise method. The other methods are also limited by the volume of water which can be sampled for viable bacteria. Typically 0.1 or 1.0 mL sample volumes are used. This source of error was recognized by Sproul et al. (1979) and is reflected by the scatter in their data. The pour-plate procedure has an additional disadvantage in that disinfection-stressed bacteria are also subjected to thermal shock which may inhibit damaged organisms thereby underestimating viable bacteria. E. coli density in the study reported here was estimated from a minimum of two replicate samples per trial. Sample volumes ranged from 10^{-4} to 800 mL depending on ozone dose. MF procedures were followed meticulously, yet it was found that viable bacteria were not always distributed as predicted by the Poisson distribution (Fisher et al., 1922). This suggested caution is required for reliable estimates of viable bacteria in disinfected water. An additional problem may occur if the underlying probability distribution is not correct. Alternative distributions may apply such as the negative binomial distribution discussed by Haas and Heller (1986).

The method for determining ozone in aqueous solution is important for reliable control of ozone disinfection. Ozone is unstable and reactive, requiring minimum handling between the sampling and analysis. Also ozone demand-free glassware is essential. The majority of the studies in ozone disinfection have relied upon a version of iodometry, using colorimetric or titrimetric procedures. The choice of method for analysis of ozone in aqueous solution was discussed in Chapter 1. The indigo method and arsenic-(III) direct oxidation method were recommended for determination of ozone residuals by Gordon and his coworkers (Gordon and Grunwell, 1984; Grunwell et al., 1983).

The foregoing discussion illustrates the large number of potential sources of error in ozone disinfection studies which makes comparison between studies difficult. However, one qualitative conclusion is evident regardless of the study: very small amounts of ozone can quickly inactivate large numbers of E. coli in the absence of competing ozone demand.

6.4.4 Practical Importance of the Results

While it is interesting to investigate the response of a high concentration of E. coli over a wide range of ozone doses, the practical disinfection region in wastewater disinfection is from 99 to 99.99% removal of E. coli. Considering Figure 6.5, it can be seen that an approximately log-log response can be used to model the dose-response from 0 to 99.99% removal. The most useful model will consider the data where the reaction rate is essentially zero indicating that the reaction is approximately complete or where the rate is very slow.

The dose-response model described by [4.2] was selected and the parameters estimated by linear least-squares using data from utilized ozone doses of 4.4 to 13 μg/L for 120 s contact time (Table 6.1). The resulting model was:

$$\log\frac{N}{N_0} = -0.681 - 3.98 \log C_U \qquad [6.7]$$

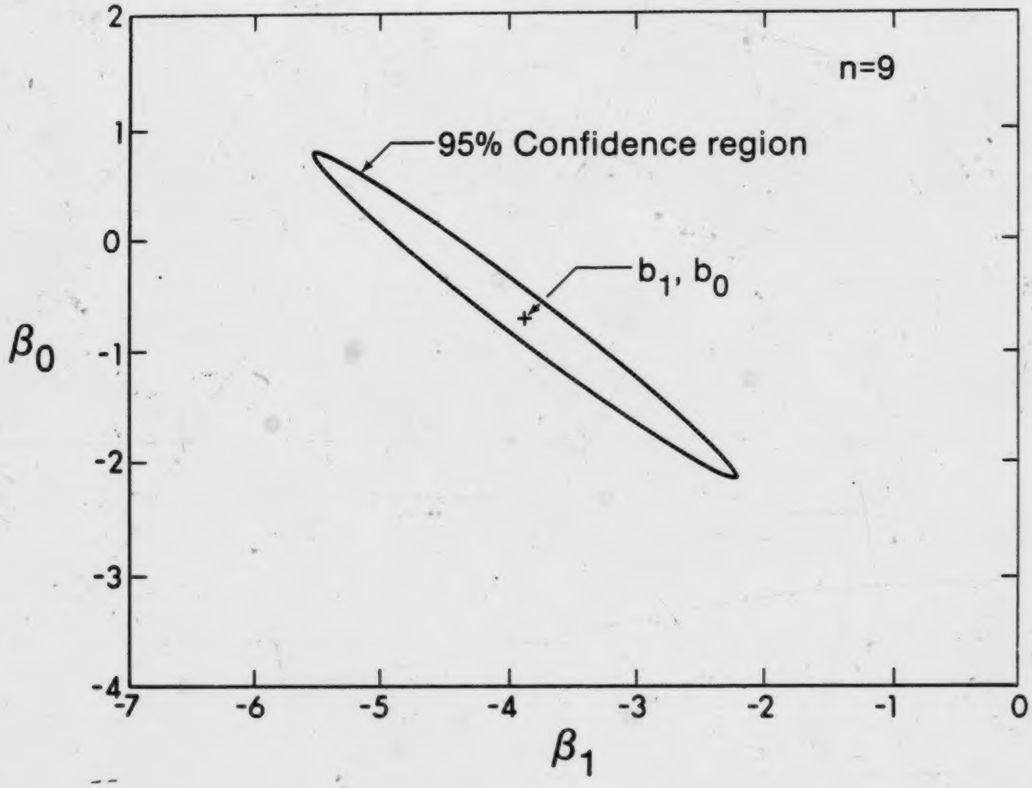
The R^2 value was 0.87 and the no-kill intercept was 0.67 μg/L of utilized ozone. Examination of the residuals and comparing the experimental error mean square with the residual mean square revealed no lack-of-fit in the experimental region used ($Pr \leq 0.01$). Details of the regression ANOVA are provided in Appendix K. Since the model was adequate, the individual 95% confidence limits for the parameters were:

$$-1.88 \leq \beta_0 \leq 0.513$$

$$-5.36 \leq \beta_1 \leq -2.60$$

This same model also describes the response of E. coli to applied ozone in the region where ozone residual is not detectable. The 95% joint confidence region for β_0 and β_1 is plotted in Figure 6.8. The long, elliptical shape is similar to that in Figure 4.3 where it was found that the two parameters were highly correlated. Therefore, care must be taken when comparing these parameter estimates with others that have been published. However, it is interesting to note that the estimate of the parameter associated with utilized ozone dose (β_1) compared favourably with that reported in the literature for fæcal coliform organisms (Caverson et al., 1986; Rakness et al., 1986). This suggests that the utilized ozone dose is the most important criterion for predicting disinfection performance and that the parameter associated with ozone dose may be independent of the wastewater strength and scale or type of reactor vessel.

FIGURE 6.8 95% joint confidence region for the dose-response model parameters, β_0 and β_1, obtained from ozone disinfection data for E. coli in ozone demand-free phosphate buffer.



6.5 CONCLUSIONS

The results of this study demonstrated that greater than 99.9% reduction of E. coli could be achieved when using an applied ozone dose of 4.4 μg/L with a contact time of less than 60 s in the absence of ozone-demanding substances. At applied ozone doses of 4.4 to 45 μg/L, there was evidence indicating that the ozone concentration was too low for rapid inactivation of E. coli. Whereas at intermediate ozone doses and moderate E. coli reductions, there was a detectable reaction rate. Further research is needed to determine the reaction order and kinetic constants of the disinfection reaction.

Published empirical dose-response models for wastewater disinfection literature were inadequate for describing the entire dose-response region. However over the region of practical interest, 99 to 99.99% removals, an empirical model using the utilized ozone dose was found to be adequate for a contact time of 120 s. The choice of data used for estimating the model parameters greatly influenced the parameter estimates and a procedure for choosing data for inclusion is needed.

The most effective disinfection region in terms of ozone utilization for disinfection occurred before an ozone residual was detectable.

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7. THE DOSE-RESPONSE OF E. COLI IN SECONDARY EFFLUENT

7.1 INTRODUCTION

The results presented in Chapter 6 indicated that the parameter esimate associated with ozone dose, may be a function of the micoorganism and may not be directly influenced by the wastewater strength or the type and scale of chemical reactor. Therefore, it is believed that a series of experiments similar in concept to those of Chapter 6, would provide insight into the relationship between the strength of the wastewater and the scale and type of reactor. This information is of interest in ozone process design for wastewater disinfection applications.

The ozonation system can be broken down into four broad areas of engineering (United States EPA, 1986):

1. process design;
2. gas preparation and ozone generation equipment;
3. gas-liquid reactor design and associated destruction of ozone in the off-gas; and
4. process control.

The second area consists largely of specifying "off the shelf" equipment provided by specialist manufacturers. The design input for these items concerns the sizing and performance specification of individual components. However, the other three areas are often the responsibility of the consulting engineer. The United States EPA has sponsored significant research programs into gas-liquid reactor design and process control methods (Bollyky and Siegal, 1977; Kirk et al., 1972; Meckes et al., 1983; Nebel et al., 1973; Rakness et al., 1984; Rosen et al., 1975; Stover and Jarnis, 1980; Venosa and Meckes, 1983; Venosa et al., 1979a, 1979b, 1980, 1985). The knowledge acquired during the research program into ozone wastewater disinfection has been summarized in a practical design manual produced by the United States EPA (1986). The Water Pollution Control Federation has also published an updated version of their Manual of Practice on wastewater disinfection which summarizes the state-of-the-art in wastewater disinfection using ozone and other processes (WPCF, 1986). The WPCF handbook provides good background information on ozone disinfection in general but it is not sufficiently detailed to provide assistance in practical process design. This is particularly true in the discussion of contactor design which is of theoretical interest, but has little practical design information. Whereas the EPA manual provided detailed design information relevant to the evaluation and design of systems for effective ozone wastewater disinfection.

Of direct importance in process design is the question of technical and economical feasibility of the ozonation process. The EPA design manual discusses the use of pilot-plants and disinfectant dose-bacteria response relationships for process design. However, full-scale plants often fail to perform according to the pilot-scale dose-response relationships. Some reasons which have been postulated for the failure of full-scale plants to perform as expected include (Rakness _et al_., 1986a):

1. short-circuiting in the contactor;
2. inadequate mixing; and
3. differences in retention time.

The dose-response function usually has one independent variable, ozone dose. The parameter estimate for this variable is thought to be a function of the wastewater strength and the configuration of the ozone contactor. The general form of the empirical model is:

$$\log\frac{N}{N_0} = \beta_0 + \beta_1 \log C_{ozone} \qquad [7.1]$$

where N is the concentration of surviving bacteria, N_0 is the initial concentration of bacteria, C is the ozone dose, and β_0 and β_1 are model parameters.

Caverson et al. (1986) cited models with one or two independent variables which had been used to describe ozone disinfection data from wastewater. Two-variable models included a third parameter associated with wastewater strength such as chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD_5), or total organic carbon (TOC) (WPCF, 1984). Typically the parameters have been estimated by the method of linear least-squares, also know as multiple-regression analysis (WPCF, 1984). However, investigators have rarely reported checking the validity of the underlying assumptions of the method of least-squares. Many of the dose-response studies found in the literature involved pilot-scale or full-scale investigations of continuous flow systems (for example; Caverson et al., 1986; Given and Smith, 1979; Meckes et al., 1983; Stover and Jarnis, 1981). Typically, these systems were operated under various conditions of ozone dosage, retention time, gas-liquid flow ratio, ozone concentration in the feed-gas, and wastewater quality. Disinfection performance was evaluated by determining the surviving coliform density based on single grab samples using standard bacteriological methods. This resulted in the collection of a large quantity of disinfection data which was used to estimate the parameters for model [7.1]. Box et al. (1978) summarized the hazards of using happenstance data to produce a cause-effect model. These included:

1. inconsistent data such as may occur when instrumentation calibration drifts or wastewater characteristics change;
2. the experimental region examined may be too small relative to the magnitude of the effect;
3. semi-confounding of effects as a result of unmeasured changes in conditions such as temperature or wastewater strength which alters the ozone demand conditions; and
4. nonsense correlations between a dependent random variable and an independent variable where, in fact, there is no causal relationship.

If the underlying assumptions of the method of least-squares are violated and happenstance data are used to obtain the model parameter estimates, errors may be introduced into the parameter estimates (Box et al., 1978; Draper and Smith, 1981). Venosa and his coworkers made the best effort at adequate experimental design of all the ozone wastewater disinfection studies in the literature (Meckes et al., 1983; Venosa et al., 1980). They accounted for the effects of changing wastewater characteristics by use of statistical experimental design and analysis. Also, they carefully controlled the values of the independent variable, applied dose, enabling comparisons to be made amongst wastewaters with different water qualities and reactor conditions.

The evaluation of a process at pilot-scale necessarily constrains the investigator to the conditions imposed by the design of the pilot-plant. For feasibility studies, this is inefficient for the purpose of discovering if a process can achieve the treatment objectives. Schmidtke and Smith (1983) noted that bench-scale testing was valuable for the following purposes:

1. establishing process feasibility;
2. developing basic design relationships; and
3. defining the range of performance within and beyond the expected experimental region.

Since there is a paucity of published bench-scale data on ozone wastewater disinfection in the literature, a bench-scale study was undertaken to determine the basic dose-response relationship of a typical fæcal indicator organism, Escherichia coli, under conditions which were controlled as far as possible with respect to the source of wastewater, temperature, organism, and ozone dose. The results of this study could then be compared with the results presented in Chapter 6, to determine if any differences in the dose-response function could be attributed to the change in the water type.

To test if factors of scale indeed exist, the second part of this study investigated the scale-up effects from a bench-scale batch reactor to a pilot-scale, semi-batch stirred tank reactor (BSTR). Robson (1982) described a typical bench-scale reactor. This was a 1.0 L gas absorption flask with a fritted glass fitting for distributing the gas bubbles in the flask and for mixing. Operated in the semi-batch mode, this apparatus permits preliminary dose-response plots to be produced for the process. However, there are some limitations using this type of apparatus. The small volume of test liquid and the necessity for precise feed-gas and off-gas ozone measurements are potential sources of error in the calculation of the ozone dose requirements. This method may have its uses in preliminary investigations establishing first approximations of ozone demand, but it has little versatility for detailed study. Consequently pilot-scale systems are often used to determine the feasibility and design parameters of the ozone disinfection process. Unfortunately, little regard is usually given to the likely prototype reactor to be used, resulting in unsatisfactory performance of the prototype because of changes in the scale.

A larger volume of liquid in a reactor which can be readily scaled-up, provides the best alternative to small gas-scrubbing flasks. The use of a stirred tank reactor operated in a semi-batch mode provides the opportunity for promoting good mass transfer of ozone in a system which is mass transfer-limited plus has the additional benefit of flexibility in mixing conditions (Gurol, 1985; Masschelein, 1982b). An additional benefit is derived from the fact that process kinetics for a batch reactor are identical to those in ideal plug flow reactors and can be approximated by a series of complete mix reactors (Grady and Lim, 1980).

This study had four objectives:

1. to determine if the dose-response relationship in secondary effluent was similar to that for ozone demand-free water;

2. to determine if the dose-response of E. coli could be modelled using the empirical model form commonly used for process design in ozone wastewater disinfection systems;
3. to determine if there was a difference in disinfection performance at bench-scale when compared to pilot-scale; and
4. to determine if the mixing intensity of a gas-liquid ozone contactor affected the disinfection performance.

7.2 MATERIALS AND METHODS

7.2.1 Wastewater Sampling and Characteristics

7.2.1.1 Bench-scale experiments

The wastewater used in the bench-scale study was collected from the City of Edmonton's Gold Bar Wastewater Treatment Plant. Twelve litre, 24 h composite samples were collected using an automatic, refrigerated (4° C), sampler on a flow basis. The 24 h period was measured from 0700 to 0700 h. Wastewater analyses were performed by the City's laboratory using standard methods (APHA, 1985). The characteristics of the three batches of wastewater used in the bench-scale experiments are summarized in Table 7.1. Temperature refers to the temperature of the wastewater at the time of the experiment. The water quality values in Table 7.1 are 70% of those reported by the City of Edmonton since 7:10 dilutions were achieved as a result of the experimental protocol for the bench-scale tests.

Composite samples were used to reduce the effect of hourly variations in wastewater quality. Otherwise atypical wastewater characteristics may bias the outcome of the experiments.

TABLE 7.1 Characteristics of secondary wastewater used in the bench and pilot-scale ozone disinfection experiments.

Scale	Date collected	Run no.	COD, mg/L	BOD_5, mg/L	TOC, mg/L	HCO_3^-, mg/L	pH	TSS, mg/L	Temperature, °C
Bench‡	20.05.87	1	29	14	-	130	6.0	6	18
	21.05.87	2	35	22	-	170	6.2	5	19
	26.05.87	3	34	20	-	170	6.0	6	19
Pilot	09.06.87	1	-	-	-	110	6.0	3	20
	10.06.87	2	25	-	8.1	110	6.3	6	20
	11.06.87	3,4	18	-	8.6	140	6.3	3	20
	15.06.87	5,6	24	-	6.7	120	6.7	10	19
	16.06.87	7,8	10	-	8.6	130	7.6	2	20
	17.06.87	9,10	29	-	9.5	140	7.6	3	20
		$\bar{x}$	26	19	8.3	140	6.5	5	19
		SD	8.3	4.2	1.0	22	0.65	3	0.73
		CV, %	32	22	12	16	10	60	38

‡ 70% of the reported values from the City of Edmonton's analyses of the full-strength, 24 h composite samples

COD = chemical oxygen demand BOD_5 = 5-day biochemical oxygen demand TOC = total organic carbon

TSS = total suspended solids $\bar{x}$ = mean SD = standard deviation CV = coefficient of variation

7.2.1.2 Pilot-scale experiments

Six batches of secondary wastewater were used during these experiments. Each one was collected over a 20 min period at approximately 0700 h on the day of the experiment. These were transported to the University of Alberta's Environmental Engineering Laboratory where the experiments were performed. The wastewater was diluted 7:10 with deionized water for conformance with the conditions of the bench-scale experiments. The diluted wastewater characteristics were determined at the University of Alberta laboratory using standard methods (APHA, 1985). The results are tabulated in Table 7.1. Temperature refers to the temperature of the wastewater used in the experiments.

7.2.2 Ozone Equipment and Analytical Methods

7.2.2.1 Bench-scale experiments

Ozone was generated using the same apparatus as in Chapter 5. Ozone concentration was consistently 5.4% w/w at ambient conditions of temperature and pressure. Oxygen flow-rate was 80 L/h at ambient temperature and pressure. The ozone-oxygen gas mixture was bubbled for a minimum of 40 min through 400 mL of 5° C MILLI-Q® water (Millipore Corp.) in a 500 mL gas absorption flask immersed in a recirculating cold water bath. The stock ozone solution concentration ranged from 27 to 32 mg/L using this procedure.

The concentration of ozone in aqueous solution was determined using the indigo procedure described in Chapter 6.

7.2.2.2 Pilot-scale experiments

The semi-batch stirred tank reactor was the same as was used in Chapters 3 and 4 (Figure 3.1). Ozone was generated using the same apparatus as in Chapter 5. As in Chapter 4, feed-gas and off-gas ozone concentrations were determined using ultraviolet spectrophotometry which was periodically checked by the iodometric procedure of Birdsall et al. (1952). Residuals were determined using the indigo method (Bader and Hoigné, 1982). Ozone concentration in the feed-gas was approximately 57.9 mg/L (STP) and the feed-gas flow-rate was 132 L/h (STP) for all of the experimental trials.

The back pressure on the ozonation system was maintained at 21 kPa to simulate conditions of an actual reactor.

7.2.3 Bacteriological Methods

The bacteriological methods were identical to those described in Chapter 5 except that the TLPY growth medium contained nalidixic acid (100 μg/mL) to inhibit lactose-fermenting bacteria other than the nalidixic acid-resistant strain of E. coli which was seeded in the secondary wastewater.

7.2.4 Procedures

7.2.4.1 Bench-scale experiments

All glassware was rendered ozone demand-free using the methods described in Chapter 5. Experiments were performed randomly using four dose levels examined at contact times of 60 and 600 s. The mean applied ozone dose levels were 9.4, 4.9, 2.4, and 1.3 mg/L. A single batch of secondary effluent was used for each set of randomized trials. The maximum ozone dose level of 9.4 mg/L required a 300 mL volume of stock ozone solution, therefore, 700 mL of secondary effluent was added to a 2000 mL Erlenmeyer flask which upon addition of the ozone stock solution would produce a final volume of 1000 mL. For lesser doses, an appropriate volume of ozone demand-free MILLI-Q® water was added to the wastewater before adding the ozone solution. Therefore, a 7:10 dilution of secondary effluent was accomplished.

The procedure for the balance of the experiment was identical to that described in Chapter 6. Prior to adding the sodium thiosulphate, a 90 mL portion of the ozonated solution was added to the prepared indigo reagent for ozone residual analysis. As in Chapter 6, the applied dose in these trials was analogous to a transferred dose in systems where the ozonated gas is bubbled continuously.

7.2.4.2 Pilot-scale experiments

Prior to the start of each experiment, the ozone generator was run for a minimum of 20 min so that steady-state production of ozone was achieved. The feed-gas flow-rate was calibrated at standard conditions of temperature and pressure using a wet test meter (Precision Scientific, Inc.).

The agitation state of the reactor was related to the speed of the standard Rushton turbine shown in Figure 3.1. The turbine was operated at 200 RPM which corresponded to a calculated gassed power input of 255 W/m³ or a velocity gradient of 500/s. Appendix A provides the detailed calculations for the power and mixing characteristics of the stirred tank reactor.

The applied dose of ozone was calculated by:

$$C_A = \frac{C_g \cdot Q_g \cdot t_g}{V} \qquad [7.2]$$

where C_A is the applied ozone dose, C_g is the concentration of ozone in the carrier gas, Q_g is the gas flow-rate, t_g is the ozonation time, and V is the volume of the wastewater being treated. At predetermined time intervals, a 300 mL sample was withdrawn from the sampling port. Each of the predetermined times corresponded to an applied ozone dose. The ultraviolet spectrophotometer was connected to a strip chart which continuously recorded the ozone concentration in the off-gas of the reactor. Ozone residual was obtained immediately following the withdrawal of the 300 mL sample from the reactor. On the average, sampling took a total of 30 s. Since the total time of ozonation was approximately 17 min for the highest dose of ozone, the contact time chosen for these experiments was 22 min. This permitted all of the samples to sit in quiescent conditions for a minimum of 5 min. At the end of 22 min, all samples were treated with excess sodium thiosulphate to stop the oxidation reactions. Bacteriological analysis was completed within 40 min.

7.3 RESULTS AND DISCUSSION

7.3.1 Bench-scale Experiments

Data collected from the bench-scale dose-response trials is tabulated in Appendix G. One-way analysis of variance (ANOVA) of the response ($\log\frac{N}{N_0}$) with respect to contact time revealed that there was no significant effect

($Pr \leq 0.01$) attributable to contact time. Therefore the results were analysed by pooling the observed responses at each dose level, independent of contact time, and considering only the effects of dose on the survival of E. coli. Since the variance was approximately constant throughout the range of doses tested, the observations were pooled to estimate the error variance. The pooled estimate of error variance was 0.0161 with 15 degrees of freedom and was used to determine the 95% confidence limits for the mean response at each dose level independent of contact time. The data gathered at each dose level are tabulated in Table 7.2 and are graphically presented in Figure 7.1. An ozone residual was detectable only after the shortest contact time, 60 s, and at the highest applied dose, 9.4 mg/L. Otherwise, none was detectable by the indigo method.

It is evident from Figure 7.1 that there was a slight but significant decline of the bacteria at 1.3 and 2.4 mg/L dose levels. However, between 2.4 and 4.9 mg/L of applied ozone there was an extremely large reduction in viable E. coli (> 99.99%). Beyond 4.9 mg/L of transferred ozone there was no statistically significant decline of E. coli as shown by the overlapping 95% confidence limits of the mean response at the two highest doses. The "tailing" effect observed in the data presented in Chapter 6 (Figure 6.5) was also indicated in Figure 7.1 after 4.9 mg/L of ozone had been applied.

The ozone dose-response relationship was assumed to be described by [7.1] which, in Chapter 6, was shown to describe adequately the dose-response of E. coli for up to 99.99% reduction when using utilized ozone as the dose metameter. The data between 2.4 and 4.9 mg/L dose levels were used to estimate the model parameters using the method of least-squares since the data at the highest and lowest doses had slightly overlapping 95% confidence limits indicating that the response was not log-log linear at doses below 2.4 mg/L and above 4.9 mg/L. The resulting model was:

$$\log\frac{N}{N_o} = 4.28 - 12.5 \log C_A \qquad [7.3]$$

The R^2 value for this regression was 0.99. The data used to estimate the parameters provided the most precise estimates (Draper and Smith, 1981). However, there were no degrees of freedom available for testing lack-of-fit in the experimental region since only two levels of the independent variable were used to estimate the two parameters. Also, the narrow 95% confidence limits for

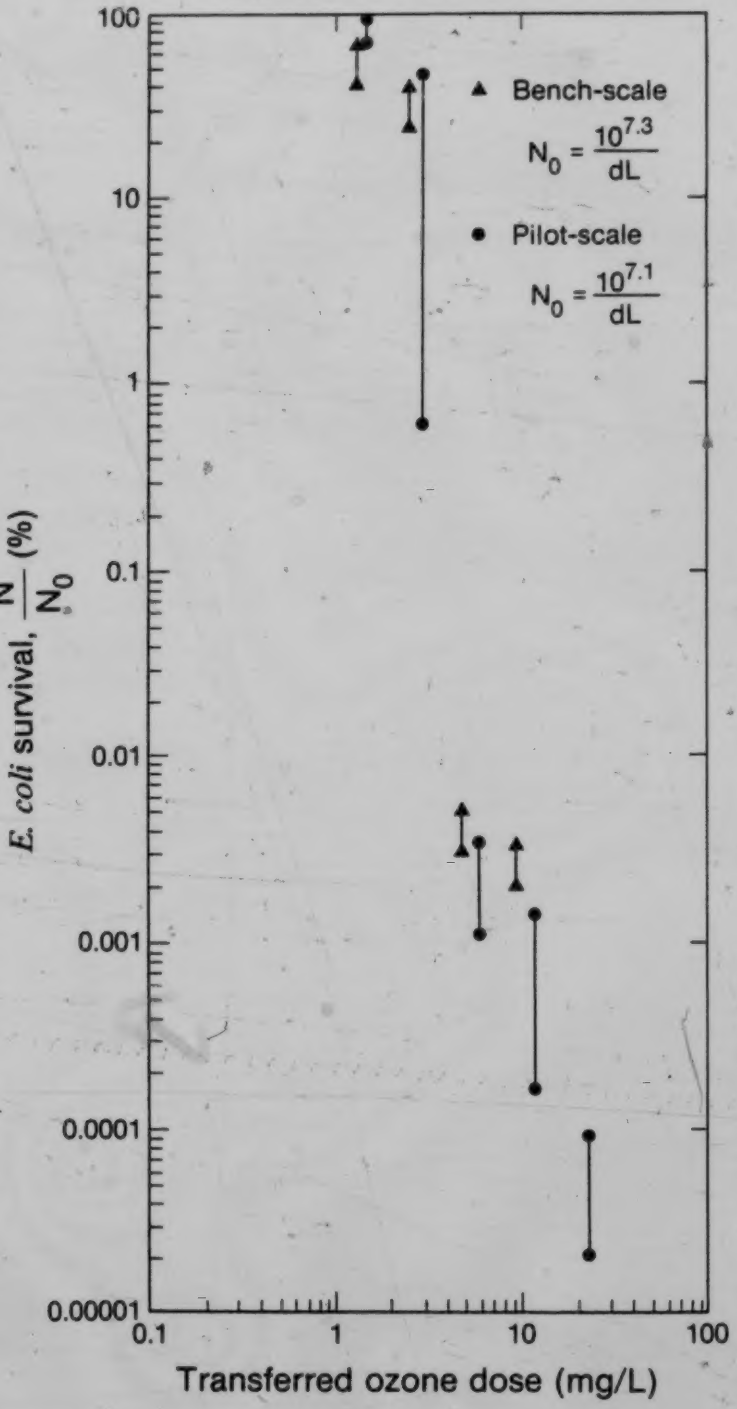
TABLE 7.2 Summary of dose-response data collected for ozone disinfection of E. coli in secondary effluent at bench and pilot-scale.

Scale	Dose level	Mean transferred ozone, mg/L	Mean utilized ozone, mg/L	Mean E. coli survival, log N/N_0	Number of trials	95% confidence limits for log N/N_0‡,†	
						Lower	Upper
Bench	1	9.4	9.4	-4.59	5	-4.71	-4.47
	2	4.9	4.9	-4.39	6	-4.50	-4.28
	3	2.4	2.4	-0.496	6	-0.605	-0.387
	4	1.3	1.3	-0.253	6	-0.362	-0.144
Pilot	1	22	21	-6.38	6	-6.71	-6.06
	2	12	11	-5.34	6	-5.81	-4.86
	3	6.0	5.7	-4.70	9	-4.94	-4.46
	4	3.0	3.0	-1.28	6	-2.22	-0.332
	5	1.5	1.5	-0.0839	6	-0.150	-0.0180

‡ Error variance for bench-scale was 0.0161 with 15 degrees of freedom

† Error variance for pilot-scale was dependent upon dose level

FIGURE 7.1 95% confidence limits for the mean E. coli response at each transferred ozone dose level for the bench and pilot-scale experiments using secondary effluent.



the response of E. coli and the use of only two settings for the independent variable provides for an R^2 value of almost one. This is not surprising since a linear model is being entertained and only two settings of the independent variable are being used to estimate the parameters. For a comprehensive explanation of the meaning of R^2, consult Draper and Smith (1981). The underlying assumptions of the method of least-squares were checked by examination of residuals and were found to be satisfied (Draper and Smith, 1981). Assuming that the model was adequate (no lack-of-fit), the individual 95% confidence limits of the parameters were:

$$3.75 \leq \beta_0 \leq 4.81$$

$$-12.8 \leq \beta_1 \leq -12.2$$

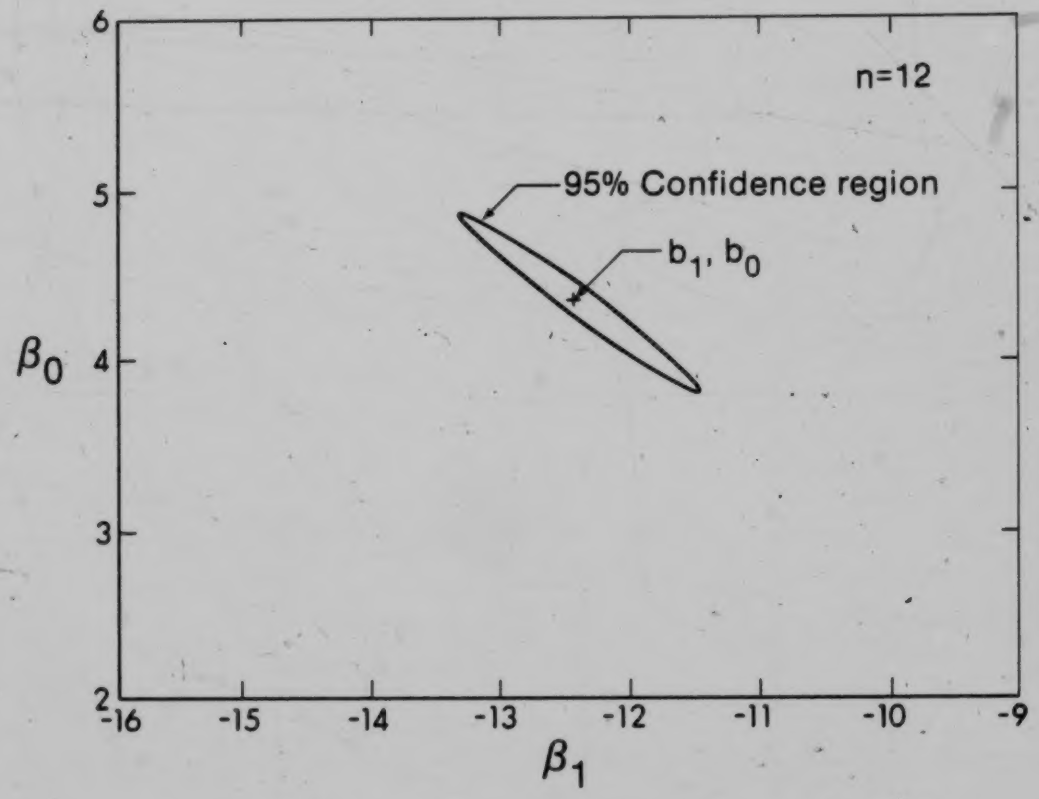
The 95% joint confidence region for the two parameters is plotted in Figure 7.2. The correlation between β_0 and β_1 is demonstrated by the long, narrow ellipse defining the region where it is reasonable to expect the true values of the parameters. The dose intercept corresponding to the no-kill condition was 2.2 mg/L of ozone which is typical of secondary effluents (Caverson et al., 1986).

Caverson et al. (1986) summarized some of the published parameter estimates and found β_1 to range from -2.5 to -4.8. Rakness et al. (1986a) reported that β_1 values obtained from the data of Meckes et al. (1983), Stover and Jarnis (1981), and Rakness et al. (1984) ranged from -2.5 to -6.7. Evidently, [7.3] does not reflect the observed dose-responses reported in the literature. This may be an artifact of the data used to estimate the parameters. When the data at all dose levels were used to estimate the parameters, the following dose-response model was obtained:

$$\log\frac{N}{N_0} = 0.743 - 5.91 \log C_A \qquad [7.4]$$

The R^2 value was 0.85 for this regression and the transferred dose intercept for the no-kill condition was 1.3 mg/L. The parameter estimate associated with ozone

FIGURE 7.2 95% joint confidence region for the dose-response model parameters, β_0 and β_1, obtained from ozone disinfection data for E. coli in secondary effluent at bench-scale.

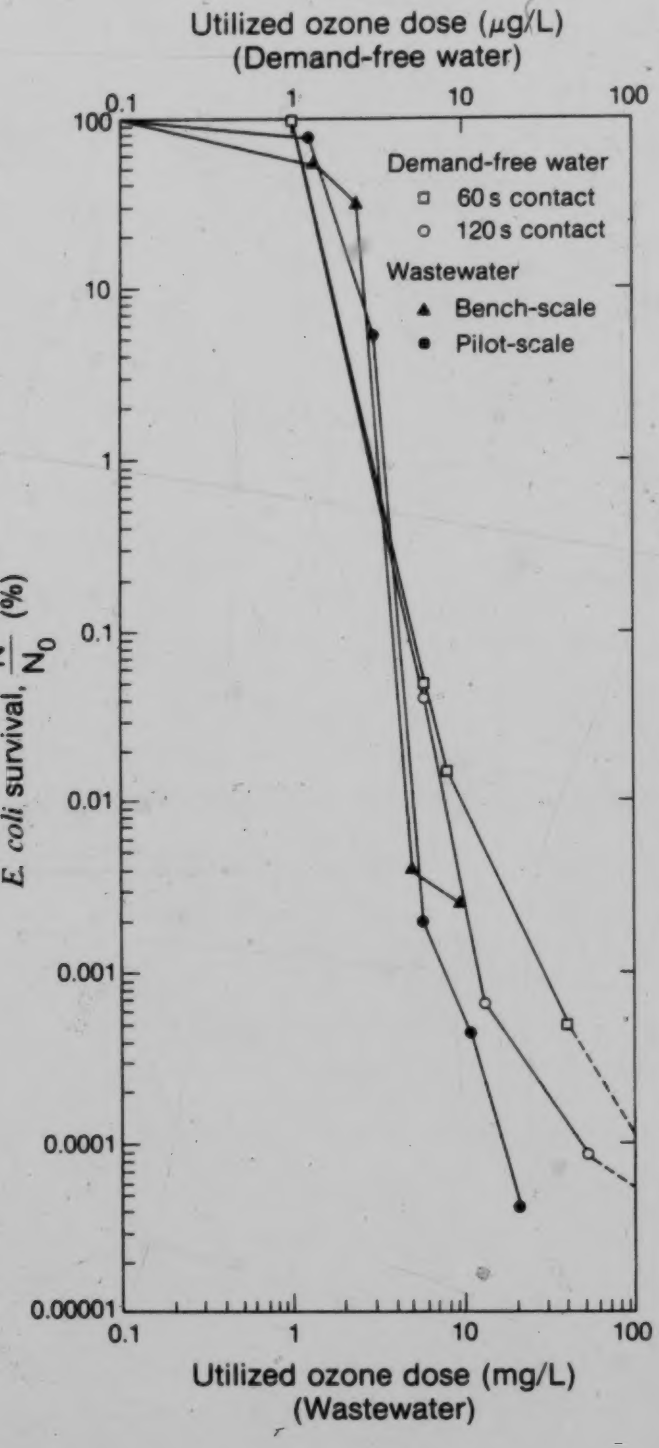


dose was in close agreement with others published for faecal coliform bacteria. However, examination of the residuals revealed that the underlying assumptions regarding the errors were not satisified. ANOVA of the regression by partitioning the error on the basis of pure error and lack-of-fit revealed that this model had significant lack-of-fit ($Pr \leq 0.01$). Details of the formal test for lack-of-fit are provided in Appendix K. Considering the lack-of-fit and the violation of the underlying assumptions of least-squares, this model was judged inappropriate for describing the dose-response of E. coli.

Models [7.3] and [7.4] illustrate the differences in the dose-response model which may occur when regression analysis is used indiscriminately. Model [7.4] superficially looks very useful, with a high R^2 value, a no-kill dose intercept which is typical of secondary wastewaters, and a dose parameter estimate for β_1 which is representative of others for secondary wastewater that have been reported in the literature. A similar argument can also be used for model [7.3] but there is a major difference between the models. The dose metameter is the logarithm of the dose. Therefore, the difference between the estimates of β_1 in models [7.3] and [7.4] result in considerable differences in the required dose to achieve the same response in E. coli. This is particularly true if the "threshold" dose is increased as a result of a water with high initial ozone demand. Thus, the choice of data to include in the regression analysis can influence the parameter estimates for the dose-response model and indiscriminate use of regression can lead to a misunderstanding of this design relationship.

Dose-response data from Chapter 6 and from this study are plotted in Figure 7.3. The bottom abscissa in Figure 7.3 is graduated in mg/L and the top abscissa is graduated in μg/L allowing convenient comparison of the ozone demand-free results with those of the wastewater. Using Figure 7.3, the ratios of the doses required for equivalent log unit reductions in the demand-free system compared with the secondary effluent system were evaluated. The mean ratio of

FIGURE 7.3 Summary of the mean responses of E. coli to utilized ozone doses in ozone demand-free water at bench-scale and in secondary effluent at bench and pilot-scale.



C_u SE : C_u PB was 960 with a coefficient of variation of 27%. This indicates that for the region of up to 99.99% E. coli reduction, approximately 960 times more ozone was required in the secondary effluent system compared with the demand-free system. The approximate initial ozone demand resulting from faster reactions than the disinfection reaction was obtained graphically and was about 1 mg/L of utilized ozone. Therefore, it is possible that the "threshold" dose is primarily a result of ozone initially consumed by competing reactions which were favoured relative to the disinfection reaction. Once sufficient ozone has been applied to the wastewater system, satisfying the competing reactions, disinfection proceeds. Evidence for this hypothesis is provided by the wastewater data in Figure 7.3, where a gradual shoulder is apparent in the E. coli response as the ozone dose increases and disinfection becomes favoured. This suggests that the disinfection reaction is occurring concurrently with competing reactions but limited ozone is available for disinfection.

The 4 log unit reduction of E. coli when increasing the ozone dose from 2.4 to 4.9 mg/L in the bench-scale wastewater experiments demonstrates that more of the applied ozone is available for disinfection. However, to achieve the same reduction in ozone demand-free water, only 6 μg/L of ozone were required. It is evident that there are competing reactions for ozone even when disinfection is apparently favoured. Therefore, the type of compounds competing for ozone in a wastewater may greatly alter the required ozone dose. This is important to recognize since a logarithmic dose metameter is used.

The experimental protocol of the bench-scale experiments limited the maximum dose to 9.4 mg/L. The mixing intensity in the bench-scale reactor was limited by the magnetic stirrer apparatus. These limitations were overcome in the pilot-scale experiments.

7.3.2 Pilot-scale Experiments

7.3.2.1 Dose-response trials at five ozone dose levels

Ozone dose-responses were investigated using six experimental trials and four batches of secondary effluent obtained on four different days. The data from the pilot-scale dose-response trials are tabulated in Appendix H. The observed response of E. coli to ozone dose is summarized in Table 7.1. The 95% confidence limits of the mean E. coli response at each dose level are plotted as a function of transferred ozone dose in and the data are shown in Figure 7.1. Transferred ozone dose in the continuously bubbled pilot reactor was analogous to applied ozone dose in the bench-scale experiments of this chapter and Chapters 5 and 6.

The mean E. coli response at each dose of ozone in the pilot experiments is plotted in Figure 7.3 as are the mean responses from the bench-scale wastewater experiments and ozone demand-free phophate buffer experiments.

ANOVA of the E. coli response indicated that there were no significant differences between batches of wastewater at dose levels 1, 2 and 4 (Pr≤0.01). However, at dose level 3, there was a difference between batches. No explanation for the difference was apparent since the measured wastewater characteristics (Table 7.1) were remarkably consistent in quality during the course of the study. The observation may be an artifact of some unmeasured quality of the wastewater such as nitrite. The ratio of ozone utilized at pilot-scale in secondary effluent with ozone utilized in demand-free water was determined. The mean ratio of Cu SE : Cu PB was 1000 with a coefficient of variation of 47%. As in the discussion for the bench-scale data reported in Figure 7.3, the interpretation of the logarithmic dose requires care since changes in the "threshold" ozone dose can seriously affect the ozone dose to achieve the desired disinfection response.

The dose-response data plotted in Figures 7.1 and 7.3 indicate that the "tailing" effect observed in Chapter 6, was also apparent in the wastewater at pilot-scale. This may be explained in terms of chemical reaction rates as discussed in Chapter 6. The rate of disinfection reaction has reduced to the point where other competing reactions become favoured. For example, it was observed consistently in each experimental trial, that the colour of the wastewater would gradually become lighter. However, after about 12 min of ozonation (approximately 14 mg/L of ozone transferred) the colour would disappear relatively quickly suggesting that the oxidation reaction for decolorization was favoured.

The parameters for model [7.1] were estimated by regression analysis. The responses at dose levels where a significant dose-response ($Pr \leq 0.05$) was observed, were used. The following model was obtained:

$$\log\frac{N}{N_0} = 0.621 - 5.72 \log C_U \qquad [7.5]$$

The R^2 value was 0.88 and the no-kill intercept was 1.3 mg/L. In spite of the apparently good description of the data on the basis of the R^2 value, residuals from the model indicated there was lack-of-fit. The shape of the residuals when plotted as a function of the predicted response at each dose level suggested that a cross-product or quadratic term was missing from the model (Draper and Smith, 1981). The lack-of-fit was caused by the tailing effect as E. coli survival decreased.

Using the E. coli survival data for the 3.0 and 6.0 mg/L transferred ozone doses, the following model was developed:

$$\log\frac{N}{N_0} = 3.80 - 11.1 \log C_U \qquad [7.6]$$

The R^2 value was 0.82 and the no-kill intercept was 2.2 mg/L of transferred ozone. There were no degrees of freedom available for testing lack-of-fit since only two dose levels were used to estimate the parameters.

Assuming the model was adequate, the individual 95% confidence limits for the parameters were:

$$1.73 \leq \beta_0 \leq 5.87$$

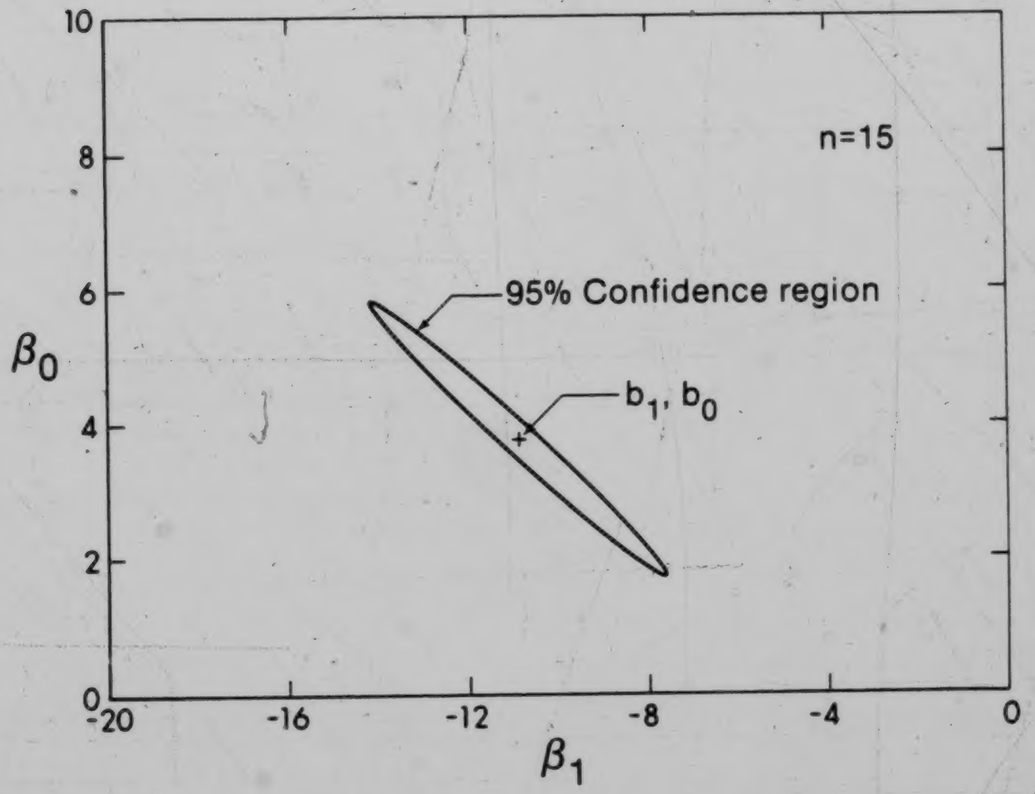
$$-14.3 \leq \beta_1 \leq -7.95$$

The 95% joint confidence region for the parameters is plotted in Figure 7.4. The correlation between the parameters is evident from the long, narrow ellipse. The parameter estimates for model [7.3] from the bench-scale investigation plot within the 95% joint confidence region obtain for the parameters using pilot-scale data shown in Figure 7.4. This indicates that for the same experimental region in bench and pilot-scales, there is no significant difference in the parameter estimates. However, it must be stressed that both of these models, [7.3] and [7.6], were developed from observations at only two settings of dose, leaving no degrees of freedom available for checking lack-of-fit. This is not a satisfactory experimental design situation.

7.3.2.2 Effect of reactor mixing conditions on disinfection

A set of experiments was conducted at 6.0 mg/L of transferred ozone to determine if mixing intensity affected disinfection performance. Data collected from this experiment are tabulated in Appendix I. The experiment was designed to test the differences in disinfection efficiency using the same BSTR and the same wastewater, but at two different power inputs. To ensure that mass transfer was not a factor, the lowest power input was the one used above (200 RPM or 255 W/m^3). The highest power input was provided by operating the Rushton turbine at 300 RPM which corresponded to a volumetric power input of 870 W/m^3 or a mean velocity gradient of 930/s. Detailed power calculations are provided in Appendix A. The experiment was conducted using the same procedures as described above, except that a single applied ozone dose of 6.0 mg/L was used rather than

FIGURE 7.4 95% joint confidence region for the dose-response model parameters, β_0 and β_1, obtained from ozone disinfection data for E. coli in secondary effluent at pilot-scale.



five. Also, an alternate sample storage condition was introduced. One sample was withdrawn from the reactor at the end of the ozonation period and was stored in the dark under quiescent conditions. The balance of the wastewater was agitated in the reactor at the same energy input as during the ozonation period but without gas flowing into the reactor. The head space of the reactor was continuously purged with air so that ozonated gas was not recirculated from the head space into the liquid. Also, any gas escaping from the liquid was removed.

At the end of 22 min, the reactor was sampled and both of the samples were treated with excess sodium thiosulphate. By means of ANOVA, no significant difference ($Pr \leq 0.01$) was observed in the disinfection response of E. coli between the two mixing conditions. However, a highly significant difference was observed between the storage conditions. There were 100 times more bacteria in the sample which had been continuously stirred for the contact period compared with the one stored in the dark, under quiescent conditions. Ozone residuals were determined by the indigo method during the course of the contact period. The quiescent sample had a detectable residual 2 min after obtaining the sample but the reactor had no detectable residual after the same time period. This indicated that the mixing conditions in the reactor promoted the removal of ozone residual. Therefore, detection of an ozone residual in the sample was associated with improved disinfection performance.

The observed difference in disinfection performance depending on the post-ozonation environment has some important implications in reactor design. Perceived differences in disinfection efficiency between types of reactors may be the result of different post-ozonation conditions. Smith et al. (1985), Stover et al. (1987), and Venosa et al. (1980) reported that stirred tank reactors had poorer disinfection performance than bubble-diffuser systems. Whereas the results of the bench and pilot-scale disinfection experiments reported in this chapter indicate that the type of reactor environment did not affect disinfection efficiency provided mass transfer of ozone was complete. Examination of the stirred tank reactor and bubble column diffuser designs reported by Smith et al. (1985) and Venosa et al. (1980) revealed some differences between reactors which may account for the differences. For instance, the stirred tank reactors in both studies had head-spaces which were continuously purged with the carrier gas. The low concentration of ozone in the off-gases, turbulent conditions in the reactor, and a relatively large surface area : depth ratio, could promote stripping of ozone from the liquid since the head-space concentration of ozone would not be in equilibrium with the concentration of ozone in solution. In contrast, the bubble columns were typically operated in series with two or three stages. These conditions, combined with a small surface area : depth ratio, could account for the reported differences in performance.

Scaccia and Rosen (1978) concluded that any well designed gas-liquid contactor could produce equivalent mass-transfer of ozone and equivalent disinfection efficiency. These observations suggest that the ozone disinfection process should be considered as two steps: a mass-transfer stage followed by a disinfection stage. This concept has been used in Europe (Masschelein, 1982a; Perrine et al., 1987).

An ozone residual was detected at the end of the 60 s contact period for the highest transferred dose in the bench-scale experiments, 9.4 mg/L. This dose level consistently achieved 99.999% reductions in E. coli. In the pilot-scale program, a 0.2 mg/L ozone residual was consistently detected when 6.0 mg/L of ozone had been transferred to the wastewater and was independent of the mixing intensity used in the reactor. Ozone residual was determined immediately, at the sampling port shown in Figure 3.1. Similarly at the higher dose levels, 12 and 22 mg/L, residuals were consistently 0.9 and 1.6 mg/L, respectively. This suggested that the assumption of a mass-transfer limited disinfection reaction may not be correct for the entire dose-response region. Much work remains to be done in the area of the chemical kinetics of ozone disinfection before these issues can be resolved. Also, the implication that an ozone residual can be detected at the end of the mass transfer period and that detection of an ozone residual was concomitant with good disinfection performance suggests that ozone residual detection may be an alternative process control parameter.

7.3.3 Dose-response in the Presence of Ozone-demanding Substances

Caverson et al. (1986), Given and Smith (1979) and Meckes et al. (1983) reported empirical dose-response models which included a term describing wastewater strength. The parameters used to quantify wastewater strength included BOD_5 and COD. The general form of the model reported by Caverson et al. (1986) was:

$$\log\frac{N}{N_0} = \beta_0 + \beta_1 \log C_U + \beta_2 \log BOD_5 \qquad [7.7]$$

The dose-response data obtained from ozone demand-free phosphate buffer used to develop model [6.7] were combined with data collected from the secondary disinfection experiments used for developing models [7.3] and [7.6]. The data were used to estimate the parameters of [7.7] by means of multiple-regression analysis. In this study, COD was substituted for BOD_5 as a measure of wastewater strength. COD in the demand-free water was assumed to be 0.001 mg/L. The mean value for COD from Table 7.1 was used for all of the wastewater observations. The resulting model was:

$$\log\frac{N}{N_0} = -5.83 - 8.88 \log C_U + 5.78 \log COD \qquad [7.8]$$

The R^2 value was 0.79. Examination of the residuals did not disclose any deviations from the underlying assumptions of regression analysis.

Assuming the model was adequate, the individual 95% confidence limits for the parameters were:

$$-6.39 \leq \beta_0 \leq -5.27$$

$$-10.6 \leq \beta_1 \leq -7.20$$

$$4.72 \leq \beta_2 \leq 6.84$$

Comparison of the parameter estimates from model [7.8] with those of Caverson et al. (1986), indicates the similarity between individual estimates of β_0 and β_2. However, there was a marked difference between the estimates of β_1. The parameters were also highly correlated, therefore the estimate of one parameter influences the estimate of the other parameters. The hazard of using empirical models indiscriminately is illustrated in the following discussion.

All of the data collected in Chapter 6 and in this study, irrespective of contact time or scale of reactor, were used to estimate the parameters of model [7.7]. The ozone demand-free water was assumed to have 0.001 mg/L of COD. The actual COD value measured for each trial (Table 7.1) was used for the wastewater data. Using 108 observations, the resulting model was:

$$\log\frac{N}{N_0} = -3.86 - 3.13 \log C_U + 2.00 \log COD \qquad [7.9]$$

The R^2 value was 0.71. Assuming the model was adequate, the individual 95% confidence limits for the parameters were:

$$-4.09 \leq \beta_0 \leq -3.63$$

$$-3.54 \leq \beta_1 \leq -2.72$$

$$1.76 \leq \beta_2 \leq 2.24$$

Examination of the residuals revealed that a cross-product or quadratic term was missing from the model (Draper and Smith, 1981). Adding the product, $\log C_U \cdot \log COD$, to the dose-response model produced the following model:

$$\log\frac{N}{N_0} = -2.26 - 4.54 \log C_U + 1.99 \log COD - 0.772 \log C_U \cdot \log COD \qquad [7.10]$$

The R^2 value for this model was 0.85, a significant improvement over that of [7.9]. Also, there was no evidence of model inadequacy when the residuals were examined according to methods described by Draper and Smith (1981). Assuming the model is adequate, the individual 95% confidence limits of the parameters are:

$$-2.63 \leq \beta_0 \leq -1.89$$

$$-4.96 \leq \beta_1 \leq -4.12$$

$$1.81 \leq \beta_2 \leq 2.17$$

$$-0.930 \leq \beta_3 \leq -0.614$$

The parameter estimates were all highly correlated.

Model [7.10] illustrates the parallel shift of the dose-response relative to utilized ozone dose indicating that the estimate of the parameter associated with ozone dose is a function of the microorganism. The interaction between ozone dose and wastewater strength illustrates the effect that the wastewater has on disinfection performance.

It is of interest that the parameter estimate for β_1 is very similar to that used by Rakness et al. (1985, 1986a) for sizing ozone equipment used for wastewater disinfection. However, the parameter estimate provides a somewhat steeper slope for the dose-response relationship than those summarized by Caverson et al. (1986). When the wastewater strength and fæcal coliform disinfection data reported in Chapter 4 and by Caverson et al. (1986), Given and Smith (1979), Rakness et al. (1984, 1985) and Smith et al. (1985) were checked with [7.10], it was found that the utilized ozone was consistently underestimated by the model. The model was statistically adequate, but was not a predictive model beyond the data reported here. A possible reason that the predicted disinfection performance was better than was observed in the studies, may be the result of a difference in susceptibility to ozone by the wild strains of fæcal coliform bacteria compared to the E. coli strain used in this study which was grown in vitro.

Evidence for this phenomenon has been published. For example, it has been reported that repeated chlorination of E. coli produced a strain which was 10 times more resistant to the effects of chlorine than the control strain (Leyval et al., 1984). Seyfried and Fraser (1980) found that a strain of Pseudomonas aeruginosa with an exocellular slime layer predominated in chlorinated swimming pool waters. The dose-response graph indicated that the slope of the response for this strain of P. aeruginosa was flatter than the other strains of P. aeruginosa which did not have a slime layer. Mitscherlich and Marth (1984), in their review of microbial survival in various environments, reported that considerable variation in heat-resistance has been observed amongst strains of the same species as well as within the strains. The age of the bacteria and the temperature at which they were grown also influenced the susceptibility to heat disinfection. They also noted that, for ultraviolet radiation (UV), E. coli were reported to be more susceptible in the logarithmic-growth phase than in the resting stage. Cells which were in the lag phase had the greatest resistance to UV radiation. Therefore, it is possible that the physiology of bacteria may have a role in determining disinfection performance when disinfecting water with chemical agents.

7.3.4 Implications for Practical Process Design

A commonly accepted condition for considering ozone disinfection of wastewater has been the requirement for extensive pilot-plant testing prior to process design (Rakness et al., 1986b; Robson, 1982). The results of this study indicate that the ozone disinfection process can be tentatively sized without the need for expensive pilot studies. This permits economical evaluation of the process without significant outlays of money. Once the process has been accepted as feasible and has been tentatively sized, pilot-plants can be designed by scaling-down the proposed prototype reactor. The pilot-plant objectives are then oriented towards optimization of process variables such as type of feed-gas, concentration of ozone in the feed-gas, gas-liquid flow ratios, mixing conditions, and process control set-points.

The type of empirical model used for sizing ozone systems used for wastewater disinfection was examined in this study. Caution must be excercised when using published models since extrapolation or interpolation can produce erroneous estimates of the required ozone dose to achieve a given reduction of bacteria. Therefore, developing the dose-response relationship for a particular wastewater, using the microorganism which will be monitored for disinfection efficiency, generally fæcal coliform bacteria or E. coli, is essential. Evidence from this study indicates that precise bench-scale experiments are useful for establishing process feasibility and preliminary sizing of the ozone system components.

7.3.5 Proposed Process Design Procedures

1. Obtain effluent water quality data from the wastewater treatment plant. The water quality parameters usually measured include COD, BOD_5, TSS, pH, temperature, TKN, nitrite, nitrate, ammonia, phosphorus, total and fæcal coliforms, metals, oil and grease, and wastewater flow-rate.
2. Determine the appropriate confidence limits for the mean value of the wastewater parameters using the historical records. Depending on the geographical location and local conditions, the data can be analysed on an annual basis, seasonal basis, or monthly basis. Statistical analysis cannot be performed using simpie statistical procedures because the data collected from wastewater effluent monitoring programs are not independent, random observations from a population of values. However, in situations where only the immediately adjacent observations are correlated (lag 1 or first-order autocorrelation), the standard error of the mean for each water quality parameter can be corrected to account for first-order autocorrelation (Box

et al., 1978; Box and Jenkins, 1976).

The standard error of the mean value of a parameter which has first-order autocorrelation is defined as:

$$SE(\bar{x}) = \frac{c\sigma^2}{n} \qquad [7.11]$$

where σ^2 is the population variance, n is the sample size used to estimate the mean, and c is the autocorrelation correction factor for the standard error of the mean. The value for c can be calculated from:

$$c = 1 + 2\frac{(n - 1)}{n}\rho_1 \qquad [7.12]$$

where ρ_1 is the lag 1 autocorrelation coefficient which can be calculated from the data (Box and Jenkins, 1976).

3. Obtain a large enough batch of wastewater for bench-scale testing and analysis of pertinent wastewater characteristics. If the characteristics lie outside the confidence region for the mean value, reject the wastewater batch as being atypical. Composite samples can help eliminate much of the hourly variation in wastewater quality and can decrease the likelihood of obtaining atypical wastewater batches.
4. Use a single batch of wastewater to examine the dose-response of fæcal coliforms and E. coli. The standard fæcal coliform MF growth medium, m-FC agar, should only be used for comparison purposes since it underestimates the number of viable bacteria surviving disinfection. The MF procedure using m-T7 agar for fæcal coliforms and modified for E. coli (Difco Laboratories, 1984) should be used for process evaluation. The amount of extra work is negligible yet more reliable disinfection data can be obtained. Samples should also be replicated at least twice and preferably three times enabling the MF procedure to be checked.
5. Replication of the experiments using the same wastewater batch can provide important information on the experimental error arising from variation in

sampling and analytical methods. Equally important is replication of the experiments using different batches of wastewater. This approach will provide information on the different sources of variation in the disinfection response. Variation from all sources can be accounted for in the confidence limits of the mean response at each dose level. Formalized hierarchal experimental designs are described by Box et al. (1978). Properly designed and analysed experiments can save much time and money.

6. The dose-response functions obtained in this manner at bench-scale can be used to size the process using the worst case scenario for wastewater quality and flow-rate and the upper confidence limits for disinfection performance.

7.4 CONCLUSIONS

The conclusions reached on the basis of the data presented here were:

1. contact time did not affect the dose-response of E. coli in secondary effluent for contact times from 1 to 22 min;
2. the model used for designing ozone disinfection systems had significant lack-of-fit ($Pr \leq 0.01$) as a result of a "tailing" effect at high ozone doses and large E. coli reduction;
3. there was no practical difference between disinfection performance of the bench-scale reactor and the pilot-scale reactor at transferred doses less than 6.0 mg/L;
4. the mixing intensity in the semi-batch, stirred tank reactor did not affect disinfection performance in the experimental region studied;
5. the detection of an ozone residual at the end of the mass-transfer stage was concomitant with significant E. coli reductions under quiescent, post-ozonation conditions;
6. preliminary process design can be adequately completed at bench-scale using relatively simple statistical experimental design and analysis methods;

7. the parameter estimates of the dose-response model were correlated and the values cannot be compared independently of one another; and
8. the dose-response model developed in this study is not valid for other strains of bacteria found in other waters and should not be used for design purposes.

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8. GENERAL SUMMARY AND CONCLUSIONS

8.1 GENERAL OVERVIEW

The research described in this dissertation evolved from two basic interests:

1. the effect of ozone as a disinfectant of wastewater containing antibiotic-resistant indicator bacteria; and
2. the statistical basis for designing experiments used for evaluation and sizing of the ozone wastewater disinfection process.

The study of the environmental occurrence of antibiotic-resistant coliforms in Canada was initiated by Bell and his coworkers (1980, 1981, 1983). Their work and the work of others in the previous decade (Grabow et al., 1973, 1974) suggested that wastewater treatment processes may promote the occurrence of these organisms in the aqueous environment resulting in undetermined health effects. The use of chlorine as a wastewater disinfectant had been reported to have a differential selective pressure favouring the presence of antibiotic-resistant coliforms in the final effluent (Murray et al., 1984; Rusin, 1986). Very little information was found regarding the effect of ozone on antibiotic-resistant coliforms during wastewater disinfection. Therefore, it was decided to undertake a research program which would elicit information on the effectiveness of ozone when disinfecting wastewaters containing antibiotic-resistant coliforms.

The second topic was inspired through the lack of sound statistical analysis of disinfection data collected from programs used for sizing the ozone wastewater disinfection process. The original pilot-plant studies by Given and Smith (1979) collected vast amounts of happenstance data which was subsequently fitted by regression analysis to an empirical dose-response model. Much of the United States EPA research into ozone wastewater disinfection used this same approach to model the wastewater disinfection process (Meckes et al., 1983; Rakness et al., 1984; Stover and Jarnis, 1981; Venosa et al., 1979a, 1979b). This approach to process design for ozone wastewater disinfection was formalized in the United States EPA (1986) design manual. However, it was felt that the process could be sized much more efficiently using well-designed bench-scale experiments. Also, there was some controversy about the reasons that the dose-response relationship changed from wastewater to wastewater and in different ozone contactors. Therefore, it was decided that a fundamental study into the factors which influence the dose-response relationship was warranted.

Throughout the experimental program, considerable time and resources were invested in development and acquisition of bacteriological procedures and data analysis techniques. The outcome of the experimental program, conclusions reached, and recommendations are summarized in this final chapter.

8.2 OZONE DISINFECTION OF MAR E. COLI

8.2.1 Promotion of MAR E. coli in an Activated Sludge Effluent

Measuring the occurrence of antibiotic-resistant bacteria in various aqueous environments is difficult and requires a large commitment of resources to delineate properly the extent of the problem and its significance to public health. The large degree of variation in both the raw and treated wastewater quality can cause difficulty in determining statistically significant differences between the influent and effluent proportions of MAR E. coli, particularly if the differences are small compared with the variance of the data. Larger sample sizes can help overcome this difficulty but these can quickly overwhelm laboratory resources. This study utilized an experimental design technique based on paired observations of the influent and effluent proportions of MAR E. coli which helped to eliminate the effect of day-to-day variation of the wastewater.

The data which were collected indicated that the Gold Bar activated sludge plant promoted a greater proportion of MAR E. coli in the treated wastewater when compared with the raw wastewater. The overall reduction of E. coli through the treatment works was approximately 90% which is typical of an activated sludge treatment system.

Enhanced resistance to environmental stress by MAR E. coli has been found by others to be an unlikely reason for increased proportions of MAR indicator bacteria in final effluents. Several investigations have found that quiescent conditions in secondary and primary clarifiers permits the transfer of resistance plasmids amongst bacteria. This is most likely the reason for the observations at the Gold Bar plant especially in light of the operating procedure of recycling waste sludge from the secondary clarifiers to the primary clarifiers.

8.2.2 Control of MAR E. coli in Wastewater Discharges by Ozone Disinfection

Ozone disinfection had no selectivity for MAR E. coli contrary to reports by others that differential selection occurred when using chlorine and ultraviolet radiation.

The investigation of the response of MAR E. coli at various ozone doses was prompted by reports of the "all or none" response of bacteria disinfected by ozone. It was postulated that if ozone caused sub-lethal injury in bacteria, a differential selection of MAR E. coli may occur as a result of a plasmid-mediated resistance to the disinfectant. Examination of the median value of the $\frac{P_i}{P_0}$ ratio for each MAR class revealed that there was a lack of differential selectivity by ozone at all of the doses used. This was consistent with the results of the single dose experiments which indicated that ozone indiscriminately inactivated MAR E. coli.

During the course of this investigation it was found that the ozone dose-response function normally used to design ozone wastewater disinfection processes had lack-of-fit. Reasons for the lack-of-fit were not clear since the experiments were not designed in such a way as to permit evaluation of the factors affecting the dose-response relationship. It was evident that an investigation into the dose-response of a specific strain of coliform bacterium may provide some insight into the reasons for the lack-of-fit of the typical design model.

8.3 THE DOSE-RESPONSE OF E. COLI DURING OZONE DISINFECTION OF WATER AND WASTEWATER

To control as many of the potential sources of error as possible in determining the dose-response relationship of a typical coliform bacterium, the following considerations were important:

1. the indicator organism should be a known strain;
2. the indicator organism should be recoverable from mixed populations of bacteria;
3. the method for enumerating the organism should recover all viable organisms including sub-lethally injured ones;
4. the dose-response of the indicator organism in an ozone demand-free system should be documented as a bench-mark for comparing the dose-response in other waters;
5. the experiments should be designed to provide randomized, independent observations of the response to different levels of dose and contact time;
6. wastewater treatment experiments should utilize one batch of wastewater per set of treatment levels; and
7. sufficient true replication should be performed to determine precisely the mean response at each dose level.

8.3.1 Enumerating Ozone Injured E. coli in Water

Sub-lethally injured indicator bacteria occur in chlorinated water and wastewater. The degree of disinfection has been found to be overestimated since the selective growth media and incubation temperatures normally used in sanitary microbiology inhibit the growth of sub-lethally injured coliform bacteria. While data regarding this phenomenon was relatively complete for chlorinated waters, the recovery of sub-lethally injured organisms from ozone treated waters was not well documented. The intention of this research was to determine if the standard APHA (1985) method for fæcal coliform enumeration was suitable for ozone treated waters.

It was found through a series of paired experiments using a known strain of E. coli (ATCC® 11775), that the standard method recovered less than half of the viable surviving organisms when compared with a non-selective growth medium. Therefore, the use of the standard APHA method biases the disinfection performance of a process in favour of the process. It was concluded that a non-inhibitory growth medium such as m-T7 (Difco Laboratories, Inc.), should be used to evaluate the disinfection performance of ozone in water and wastewater.

8.3.2 The Dose-response of E. coli in Demand-free Water

The intention of the investigation using demand-free water was to determine the mean response of E. coli at predetermined ozone dose levels. By using replicated, randomized, independent batch experiments, precise estimates of the response could be obtained. Also, the dose-response models reported in the literature could be evaluated. Particular care was taken in preparing the bacteria and glassware to eliminate effects caused by extraneous ozone demand.

The results indicated that E. coli were very sensitive to ozone and very small doses produced significant inactivation. For instance, 99.95% removal was obtained with 4.4 μg/L of ozone at contact times of less than 60 s. However, the dose-response models used for sizing the ozone wastewater disinfection process had significant lack-of-fit and were inadequate.

Contact time was found to have some effect for intermediate doses of ozone, but the effects were small relative to those associated with the utilized ozone dose. It was postulated that the observed dose-response could be explained by chemical kinetic theory and that the dose-response was a function of disinfection reaction rates which were dependent upon the surviving number of bacteria and the residual ozone concentration.

8.3.3 The Dose-response of E. coli in Activated Sludge Effluent

There were two principal objectives associated with the study into the dose-response of E. coli in secondary effluent. The first objective was to compare the dose-response in wastewater with that in ozone demand-free water using identical experimental methods. The second objective was related to the effects of reactor type and scale-up on disinfection performance.

Using randomized, independent trials, it was apparent that contact time had no effect on the disinfection performance for times greater than 60 s. Also, there was no practical difference between the disinfection experiments conducted at bench-scale using a concentrated ozone solution and those conducted at pilot-scale in a semi-batch stirred tank reactor.

The dose-response model recommended by the United States EPA was not adequate over the entire disinfection response which encompassed 7 log units of E. coli reduction. The choice of data to use in the estimation of the model parameters influenced the parameter estimates and the model adequacy. The impact of improper parameter estimation can be severe since the dose-response models use a logarithmic dose metameter. Changes in the initial ozone demand of a wastewater can seriously alter the ozone dose required to achieve a specified degree of disinfection. However, it was apparent that the initial 99.99% reduction of E. coli had a dose-response which could be approximated using a model form similar to the United States EPA (1986) model. It was found that there was an approximately parallel shift of the dose-response function corresponding to the ratio of "no kill" dose intercepts as measured by the COD of the water. Multiple-regression analysis of the combined disinfection data from the experiments in ozone demand-free water and in secondary effluent, revealed that there was an interaction between ozone dose and wastewater strength. Also, the model parameter estimates were strongly correlated indicating that interpretation of parameter estimates from other studies should be done with caution using the joint confidence regions.

8.4 CONCLUSIONS

The following major conclusions were reached based on the studies reported herein:

1. the activated sludge treatment process studied promotes an increase in the proportion of MAR E. coli in the final effluent when compared with the raw wastewater;
2. ozone does not differentially select MAR E. coli irrespective of dose level as has been reported for other wastewater disinfectants;
3. the standard membrane filtration procedure recommended by the APHA (1985) is not satisfactory for recovery of fæcal coliforms from ozone disinfected water;
4. detection of an ozone residual is essential for consistent disinfection performance in demand-free water and secondary effluent;
5. for the experimental region studied, contact time is not as important as ozone dose in effecting significant bacterial reductions in demand-free water and secondary effluent;
6. the parameter estimates obtained using regression analysis are highly correlated and should be considered jointly when comparing results of different studies; and
7. the type or size of reactor used in this study had no practical effect on wastewater disinfection performance in the region of practical disinfection

importance.

It was also concluded that:

1. ozone disinfection kinetics are complex and cannot be described by Chick's law;
2. utilized ozone dose is the best dose metameter for ozone dose-response relationships;
3. laboratory treatability studies are essential for the accurate determination of the correct ozone dosage; and
4. pilot-scale treatability studies are not necessary to determine the amount of ozone required to achieve a specified disinfection performance objective if properly designed experiments are used in bench-scale tests. However, pilot-scale evaluation may still be necessary for other practical aspects of process design.

8.5 RECOMMENDATIONS

A major problem with ozone disinfection of water is the absence of quantitative data regarding the reaction rates of ozone with bacteria in water. Further refinement of the ozone disinfection process depends upon information regarding the chemical reactions between ozone and microorganisms, particularly in the presence of competing oxidation reactions. A fundamental component of reaction kinetic research would include a study of the mode of action of ozone on selected indicator bacteria since the disinfection mechanism of ozone remains uncertain.

A second major area of research involves the design of ozone contactors. It is evident from the results reported here that there are basically two steps involved in ozonation: the mass-transfer stage where ozone is dissolved uniformly in aqueous solution; and the contacting stage where a minimum contact time is stipulated to ensure the opportunity for the ozone contact at the molecular level.

It is apparent that the conditions in the vessel during the contact stage should be as quiescent as possible to maximize disinfection performance.

A number of improvements could be made in the research protocol.

1. The antibiotic-resistance testing protocol can be improved by:
   a. the use of m-T7 (Difco Laboratories, Inc.) which is selective for coliform bacteria but does not inhibit sub-lethally injured coliforms. The addition of Bacto TTC (Difco Laboratories, Inc.) according to the procedure described by Difco Laboratories (1984), permits differentiation of E. coli. The use of this agar would eliminate the time (up to 24 h) required for confirmation of E. coli by the indole-spot test. In addition, it would reduce the possibility of losing resistance plasmids through spontaneous curing.
   b. More E. coli isolates should be screened for antibiotic-resistance to improve the precision of the parameter estimates for the frequency distribution.
   c. Fewer antibiotics should be investigated since twelve antibiotics required duplicates of all of the Iso-sensitest agar plates which created a large amount of extra preparation and laboratory work which was not commensurate with the additional information gained. Great care should be excercised in the selection of antibiotics.
2. Fæcal coliforms should be replaced by E. coli as the indicator organisms of choice. Recovery should be with a growth medium such as m-T7 agar (Difco Laboratories, Inc.) which has been shown to enhance the recovery of stressed coliforms and E. coli (LeChevallier et al., 1983, 1984).
3. The semi-batch stirred tank reactor would produce a more homogeneous ozone-containing off-gas if a mixing impeller was added to the head-space of the reactor.
4. The indigo method should be used for ozone residual determination in water

and wastewater based on the findings of this study and recommendations by others. Ideally, ultraviolet absorbance at 253.7 nm in flow through quartz cuvettes should be used to calibrate the procedure.

5. A visible-ultraviolet spectrophotometer is essential for analysis of ozone in aqueous solution. Also, studies involving the oxidation of organics in drinking water can be facilitated by the use of ultraviolet spectrophotometry (Edzwald *et al*., 1985). The light path requirements should be carefully assessed since the sensitivity of spectrophotometry is directly related to the light path length.
6. The concentration of ozone in the off-gas and the feed-gas should be continuously determined using ultraviolet spectrophotometers.

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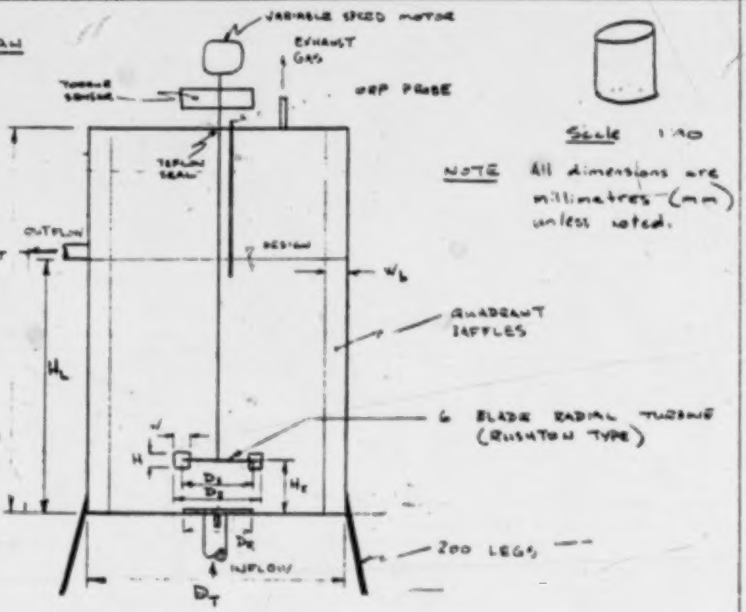
APPENDIX A: SHOP DRAWINGS AND POWER CALCULATIONS FOR THE SEMI-BATCH STIRRED TANK REACTOR

Dec 3, 1985 G.R. FINCH	CSTR OZONE WASTEWATER REACTOR	Kuo & Yocum (1982) Oldshue (1983)

OVERALL PLAN


VARIABLE SPEED MOTOR
EXHAUST GAS
TORQUE SENSOR
ORP PROBE
TEFLON SEAL
OUTFLOW
H_T
DESIGN
W_b
QUADRANT BAFFLES
H_L
6 BLADE RADIAL TURBINE (RUSHTON TYPE)
W
H
H_I
D_S
D_I
D_R
INFLOW
D_T
200 LEGS

Scale 1:10

NOTE All dimensions are millimetres (mm) unless noted.

$\frac{D_I}{D_T} = 0.34$ $\frac{D_R}{D_I} = 0.80$ $\frac{H_L}{D_T} = 1.0$ $\frac{D_S}{D_I} = 0.67$ $\frac{W_b}{D_T} = 0.10$

$D_T = 500$ ✓
$D_I = 170$ ✓
$D_S = 114$ ✓
$D_R = 136$ ✓

$H_T = 760$ ✓
$H_I = 170$ ✓
$H_L = 500$
$H = 34$ ✓

$W_b = 50$ ✓
$W = 43$ ✓

SPARGER

GAS - 0.5 holes/cm^2 ; $D = 1.5$ mm

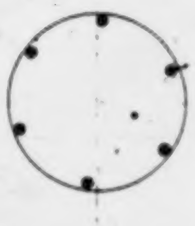
LIQUID - 40 mm dia. pipe $A = 1.26 \times 10^{-3}\ m^2$

6 small pipes, $A = \frac{1.26 \times 10^{-3}}{6} = 2.10 \times 10^{-4}\ m^2/\text{pipe}$

$\therefore D_p = 16.4$ mm (~ 3/8" dia)

DETAIL

$A_R = 1.45 \times 10^{-2}\ m^2$
$= 145\ cm^2$
∴ 72 holes are req'd

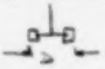


6 - 16 mm dia. TUBES

STAINLESS STEEL MESH (0.5 holes/cm^2 or 3 holes/in^2)
1.5 mm holes

Dec 6, 1985	Power Calcs for Turbine	

$$Re = \frac{D^2 N \rho}{\mu}$$



$$\mathrm{I} = \frac{Pg}{\gamma N^3 D^5} \qquad Re \geq 10^4$$

$$P = \frac{\mathrm{I} \gamma N^3 D^5}{g}$$

P = ?

$g = 9.81 \frac{m}{s^2}$

$\rho = 1000\ kg/m^3$ $\qquad \gamma = 9.81 \times 10^3 \frac{N}{m^3}$

N = 100 - 500 rpm

D = 0.170 m

$\mathrm{I} = f(Re)$

$\mu = 1.518 \times 10^{-3}\ N \cdot s/m^2$

$\nu = 1.519 \times 10^{-6}\ m^2/s$

rpm = 100

$$Re = (0.17)^2\ m^2 \cdot 100 \frac{rev}{min} \cdot \frac{1 min}{60 s} \cdot \frac{1 \quad s}{1.518 \times 10^{-6}\ m^2}$$

$$= 3.17 \times 10^4$$

rpm = 500

$Re = 1.59 \times 10^5$

rpm = 50

$Re = 1.59 \times 10^4$

All Re are in turbulent range

∴ from Rushton et al (1950) $\mathrm{I} = 7$

∴ for 100 rpm = 1.67 rev/s

$$P = 7 \times 9.81 \times 10^3 \frac{N}{m^3} \times (1.67)^3 \frac{rev^3}{s^3} \times (0.17)^5\ m^5 \times \frac{1\ s^2}{9.81\ m}$$

$$= 4.63 \frac{N \cdot m}{s} \quad \text{and} \quad \frac{N \cdot m}{s} = \frac{J}{s} = W$$

∴ = 4.63 W = 0.006 HP

for 500 rpm = 8.33 $\frac{rev}{s}$

$$P = 7 \times 9.81 \times 10^3 \times (8.33)^3 \times (0.17)^5 \cdot \frac{1}{9.81}$$

= 575 W = 0.77 HP

for 600 rpm = 10 $\frac{rev}{s}$

$$P = 7 \times 9.81 \times 10^3 \times (10)^3 \times (0.17)^5 \times \frac{1}{9.81}$$

= 994 W = 1.33 HP $\qquad$ say 1.5 HP

Torque from Oldshue (1983) pg 363

$P = \Phi \gamma N^3 D^5 g^{-1}$ $Re > 10^4$

$T = \frac{P}{2\pi N} = \frac{\Phi \gamma N^2 D^5}{2\pi g}$ $\therefore N^2 = \frac{T\, 2\pi g}{\Phi \gamma D^5}$

for 100 RPM = 1.67 $\frac{rev}{s}$

$T = 4.63 \frac{N \cdot m}{s} \times \frac{1}{1.67} \frac{s}{rev} \times \frac{1}{2\pi}$

$= 0.44$ N·m $= 3.9$ lb-in

for 500 rpm = 8.33 $\frac{rev}{s}$

$T = 575 \frac{N \cdot m}{s} \cdot \frac{1}{8.33} \frac{s}{rev} \cdot \frac{1}{2\pi}$

$= 11$ N·m $= 97$ lb-in

for 600 rpm = 10 rps

$T = 1000 \cdot \frac{1}{10} \cdot \frac{1}{2\pi}$

$= 15.9$ N·m

$= 140$ in·lb.

for Browning 133U1-LA5 E

$T_{max} = 177$ lb-in

$= 20$ N·m

174U1-LA5E

$T_{max} = 245$ lb-in

$= 28$ N·m

$N_{max} = \left(T_{max} \cdot 2\pi \cdot g \cdot \frac{1}{\Phi} \cdot \frac{1}{\gamma} \cdot \frac{1}{D^5}\right)^{1/2}$

$= \left(20 \times 2\pi \times 9.81 \times \frac{1}{7} \times \frac{1}{9.81 \times 10^3} \cdot \frac{1}{0.17^5}\right)^{1/2}$

$= 11.24 \frac{rev}{s} = 674$ rpm

Conclusion

1. 5:1 reducer will carry torque generated by 600 + rpm
2. Need minimum of 1.5 HP, 300 rpm base speed DC motor.

4-12-85	Maximum RPM Calculation	from Liquid Agitation fig 23 " 40	3

Ungassed Condition

scale of agitation from fig. 40 ~ 6 or more

from fig 25b

$v_t = 0.182$ m/s $\qquad A_T = 0.20\ m^2$

$Q = 0.182\ \frac{m}{s} \times 0.20\ m^2 = 0.0357\ \frac{m^3}{s}$

from Fig 2

$N_Q = 0.75$ and $N_Q = \frac{Q}{ND^3}$

$\therefore N = \frac{Q}{N_Q D^3} = \frac{0.0357\ m^3}{s} \cdot \frac{1}{0.75} \cdot \frac{1}{(0.170)^3 m^3} = 9.88$ rps

$= 592$ rpm

say 600 rpm max

9/11/85

PRIME MOVER AND TRANSMISSION SPECS.

POWER	1.5 HP output	max.
TORQUE	140 in-lb	max
SPEED	600 RPM	max

Dec 9, 1985	Recalculated Power, Torque, Speed for Scale-down	

Existing Equipment

Power - 0.75 HP = 560 W

Torque - 177 in-lb = 20 N·m

Speed - $350 \frac{rev}{min} = 5.83 \frac{rev}{s}$

Trial and Error Iterations - see pg 23 & 40 "Liquid Agitation"

Scale of Agitation = 6 → $V_c = 0.182$ m/s

Trial 1

Assume $T = 0.300$ m ∴ $D = 0.100$ m $\frac{D}{T} = 0.333$

$$Q_c = V_c \cdot A = 0.182 \frac{m}{s} \times \pi \times (0.1)^2 \times \frac{1}{4}$$

$$= 0.00143 \frac{m^3}{s}$$

from Fig. 2 pg 23

$N_Q = 0.75$

$$\therefore N = \frac{Q}{N_Q D^3} = 0.00143 \times \frac{1}{0.75} \times \frac{1}{(0.1)^3} = 1.91 \frac{rev}{s}$$

$$= 114 \text{ rpm}$$

check Re

$$Re = \frac{D^2 N \gamma}{\mu} = (0.1)^2 \times 1.91 \frac{rev}{s} \times .981 \times 10^3 \times \frac{1}{1.818 \times 10^{-3}}$$

$$= 1.23 \times 10^5 \therefore N_Q \text{ is OK.}$$

Trial 2

Assume $\frac{D}{T} = 0.45$ $T = 0.400$ m $D = 0.180$ m

$$\therefore Q = 0.182 \times 0.1\overline{26} = 0.02\overline{29} \ m^3/s$$

$$N = 0.0229 \times \frac{1}{0.64} \times \frac{1}{(0.18)^3} = 6.12 \frac{rev}{s} = 367 \text{ rpm.}$$

$$Re = (0.18)^2 \times 6.12 \times 9.81 \times 10^2 \times \frac{1}{1.82 \times 10^{-4}} = 1.28 \times 10^6$$

∴ N_Q is OK.

9/12/85

Trial 2 cont'd

check Power

$\Phi = 7$

$$P = 7 \times 9.81 \times 10^3 \times (6.12)^3 \times (0.180)^5 \times \frac{1}{9.81}$$

$$= 303 \ W = 0.41 \ HP$$

$$T = \frac{P}{2\pi N} = 7.88 \ N \cdot m = 70 \ in \cdot lb.$$

Trial 3

Assume $T = 0.500 \ m$ $\frac{D}{T} = 0.5$ $D = 0.250 \ mm$

$Q = 0.182 \times 0.20 = 0.0357 \frac{m^3}{s}$ $N_Q = 0.60$

$$N = 0.0357 \times \frac{1}{0.60} \times \frac{1}{(0.25)^3} = 3.81 \frac{rev}{s} = 228 \ rpm$$

$$Re = (0.25)^2 \times 3.81 \times 9.81 \times 10^3 \times \frac{1}{1.518 \times 10^{-7}}$$

$= 1.5 \times 10^6$ $\therefore N_Q$ is ok.

$$P = 7 \times 9.81 \times 10^3 \times (3.81)^3 \times \frac{1}{9.81} \times (0.25)^5$$

$$= 378 \ W = 0.51 \ HP.$$

$$T = \frac{378}{2\pi N} = 15.8 \ N \cdot m = 140 \ lb \cdot in$$

Trial 4

Assume $\frac{D}{T} = 0.45$ $T = 0.500$ $D = 0.225$

$N_Q = 0.64$

$V_0 = 0.182$ $Q = 0.182 \times 0.20 = 0.0364 \frac{m^3}{s}$

$$N = 0.0364 \times \frac{1}{0.64} \times \frac{1}{(0.225)^3} = 5 \frac{rev}{s} = 300 \ rpm.$$

check $Re = (0.225)^2 \times 5 \times 9.81 \times 10^3 \times \frac{1}{1.518 \times 10^{-7}}$

$= 1.63 \times 10^6$ $\therefore N_Q = OK$

Trial 4 cont'd

$$P = 7 \times 9.81 \times 10^3 \times 5^3 \times (0.225)^5 \times \frac{1}{9.81}$$

$$= 504 \text{ W} = 0.67 \text{ HP}$$

$$T = \frac{504}{5 \times 2 \times \pi} = 16 \text{ N m} = 140 \text{ lb-in}$$

Compare Caverson's System

$\frac{D}{T} = 0.34$ $D = 0.170$ m $T = 0.500$ m $N = 350$ rpm

$N_Q = 0.75$ $= 5.83 \frac{rev}{s}$

$$\therefore N_Q = \frac{Q}{ND^3} \quad \therefore Q = N_Q \cdot N \cdot D^3$$

$$= 0.75 \times 5.83 \times (0.17)^3$$

$$= 0.0214 \frac{m^3}{s}$$

$$\therefore v_b = \frac{0.0214}{0.196} = 0.110 \frac{m}{s} = 22 \text{ ft/s}$$

This velocity corresponds to agitation level of 3-4 which disperses bubbles but does not maximize mass transfer.

$$P = 7 \times 9.81 \times 10^3 \times (5.83)^3 \times (0.17)^5 \times \frac{1}{9.81}$$

$$= 197 \text{ W} = 0.26 \text{ HP.}$$

What is maximum scale of agitation with the new design?

$N = 350$ rpm $= 5.83$ rps.

$$Q = 0.75 \times 5.83 \times (0.225)^3 = 0.0498 \frac{m^3}{s}$$

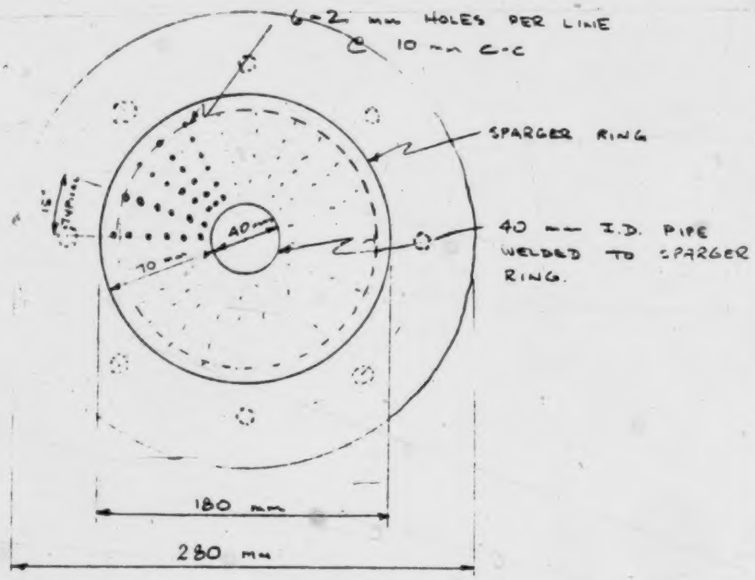
$$v_b = \frac{0.0498}{0.196} = 0.254 \frac{m}{s} = 50 \frac{ft}{s}$$

Scale of agitation is 8-9

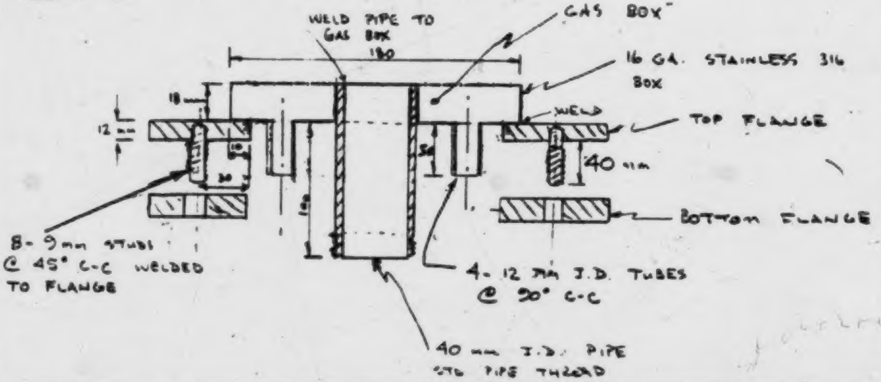
Dec 9, 1985	SPARGER ASSEMBLY DETAIL	CIVIL ENGNG 432-3950	1

GORDON FINCH

SCALE 1:3


6-2 mm HOLES PER LINE
@ 10 mm C-C
SPARGER RING
15° TYPICAL
40
70 mm
40 mm I.D. PIPE
WELDED TO SPARGER
RING.
180 mm
280 mm

NOTE. PROVIDE TEFLON GASKET, WASHERS, NUTS FOR FLANGE ASSEMBLY


WELD PIPE TO
GAS BOX
GAS BOX
180
16 GA. STAINLESS 316
BOX
18
12 mm
WELD
TOP FLANGE
10
30
50
40 mm
BOTTOM FLANGE
150
8-9 mm STUDS
@ 45° C-C WELDED
TO FLANGE
4-12 mm I.D. TUBES
@ 90° C-C
40 mm I.D. PIPE
STD PIPE THREAD

NOTE: ALL MATERIALS TO BE 316 STAINLESS STEEL FOR ALL PARTS OF APPARATUS

	Dec 9, 1985	IMPELLER DESIGN	G.R. FINCH CIVIL ENGNG 432-3950	2

D_I = 225 mm

D_D = 150 mm

w = 57 mm

h = 46 mm

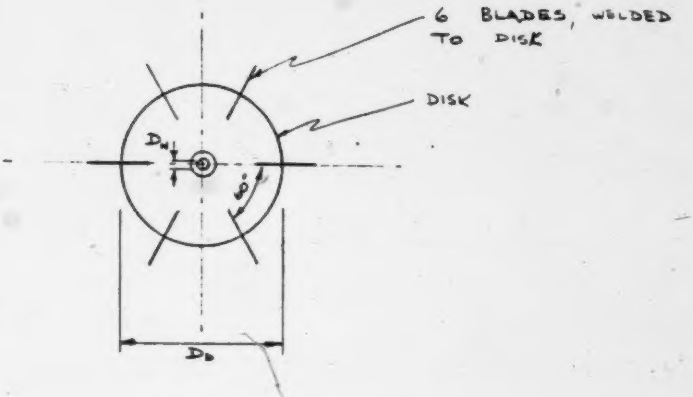
D_S = 25 mm

D_H = 12 mm

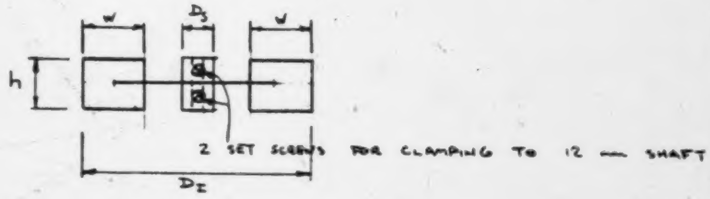
ALL MATERIAL TO BE STAINLESS STEEL GRADE 316

SCALE 1:5

TOP


6 BLADES, WELDED TO DISK
DISK
D_H
60°
D_D

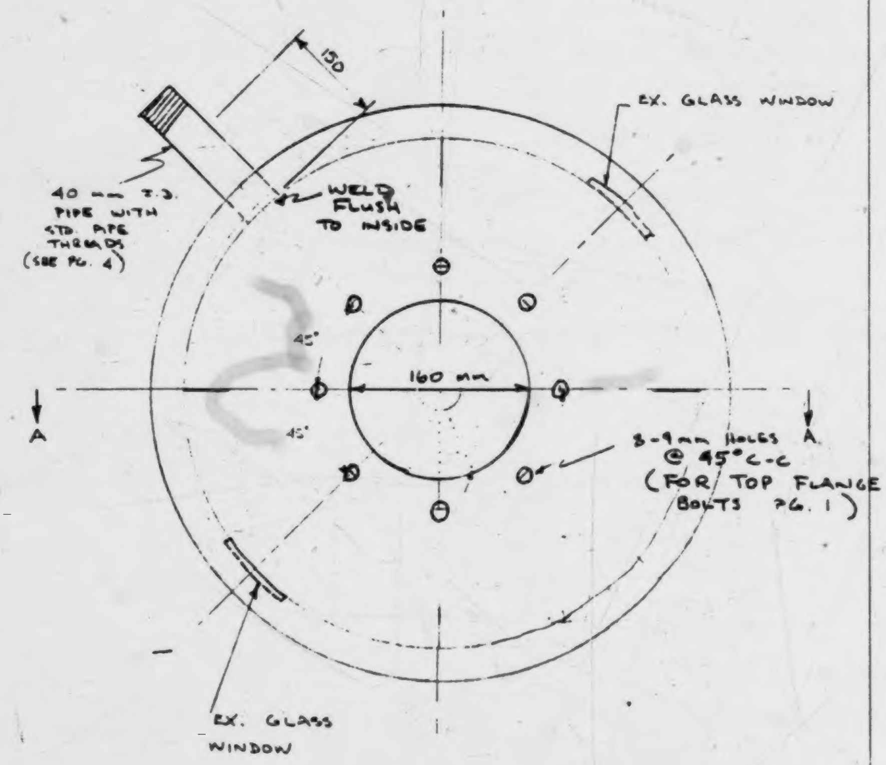
FRONT


w
D_S
w
h
2 SET SCREWS FOR CLAMPING TO 12 mm SHAFT
D_I

	9/12/85	CSTR DESIGN con'T		3

TANK DESIGN - PLAN VIEW

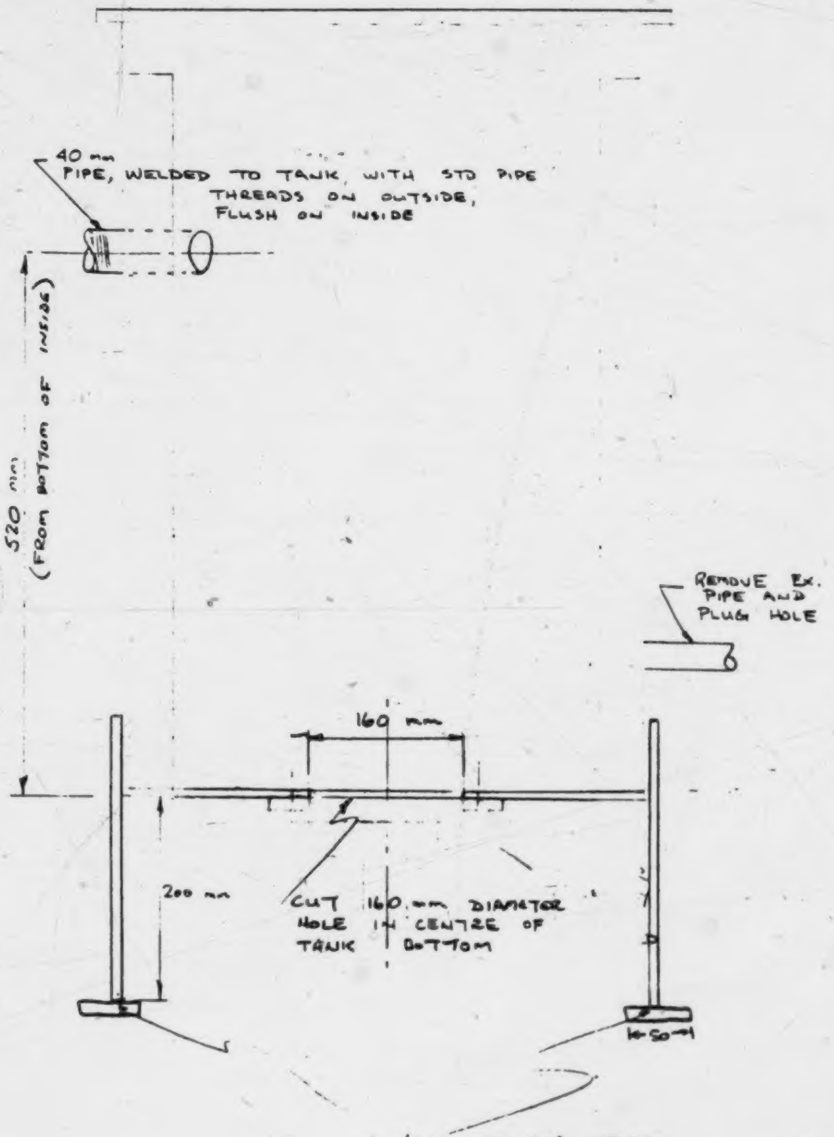
SCALE 1:5


150
EX. GLASS WINDOW
40 mm I.D.
PIPE WITH
STD. PIPE
THREADS
(SEE PG. 4)
WELD
FLUSH
TO INSIDE
45°
45°
160 mm
A
A
8-9mm HOLES
@ 45° C-C
(FOR TOP FLANGE
BOLTS PG. 1)
EX. GLASS
WINDOW

	9/12/85	CSTR DESIGN CONTINUED		4

TANK DESIGN - SECTION A-A FROM PAGE 3

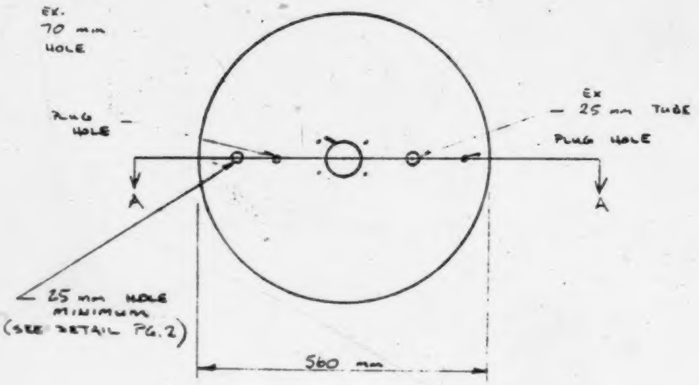
SCALE 1:5

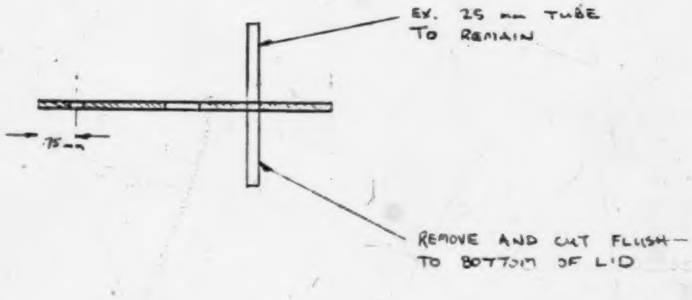

40 mm
PIPE, WELDED TO TANK, WITH STD PIPE
THREADS ON OUTSIDE,
FLUSH ON INSIDE
520 mm
(FROM BOTTOM OF INSIDE)
REMOVE EX.
PIPE AND
PLUG HOLE
160 mm
200 mm
CUT 160 mm DIAMETER
HOLE IN CENTRE OF
TANK BOTTOM
50

4 LEGS @ 90° APART.

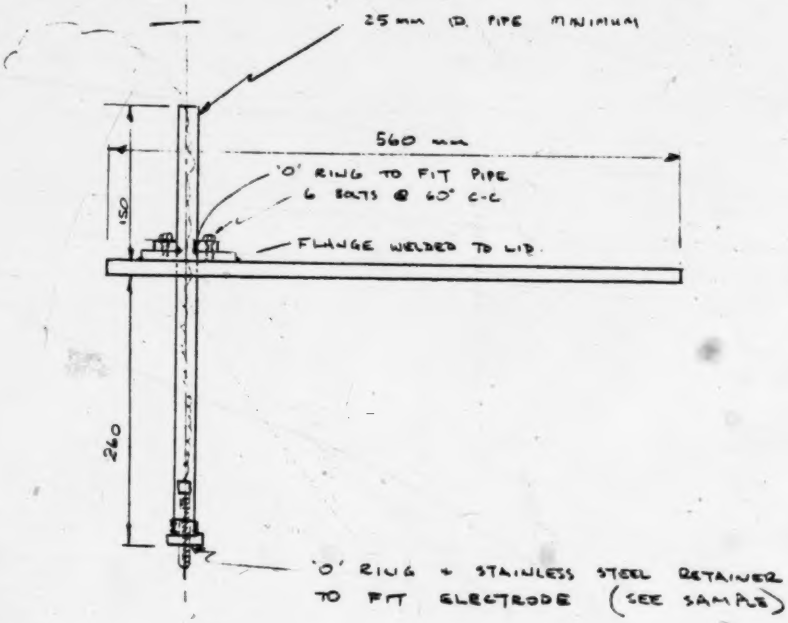
11-12-85 G.R. FINCH	REACTOR TANK TOP		1

SCALE 1:10


EX. 70 mm HOLE
PLUG HOLE
EX 25 mm TUBE
PLUG HOLE
A
A
25 mm HOLE MINIMUM (SEE DETAIL PG.2)
560 mm


EX. 25 mm TUBE TO REMAIN
75 mm
REMOVE AND CUT FLUSH TO BOTTOM OF LID

11-12-85 G.R. FINCH	ORP PROBE ASSEMBLY DETAIL		2


25 mm ID. PIPE MINIMUM
560 mm
'O' RING TO FIT PIPE
6 BOLTS @ 60° C-C
FLANGE WELDED TO LID.
150
260
'O' RING + STAINLESS STEEL RETAINER TO FIT ELECTRODE (SEE SAMPLE)

NOTE:

1. 'O' RINGS TO BE TEFLON
2. STEEL TO BE S.S. 316

June 3, 1987 | Characterization of | BSTR Power Characteristics

Reference: WPCF 1986. Wastewater Disinfection.
MOP FD-10 WPCF, Alexandria, VA.
pp. 110-112.

1. Ungassed State (20°C)

$$Re = \frac{d_I^2 \, N \, \rho_L}{\mu_L}$$

$$= \frac{d_I^2 \, N}{\nu_L}$$

$\nu = 1.003 \times 10^{-6} \frac{m^2}{s}$

$N = 2.00, 3.33, 5.00 \frac{rev}{s}$

$d_I = 0.170$ m

(a) N = 2.00 rev/s = 120 RPM

$$Re = \frac{0.170^2 \, m^2 \times 2 \, rev/s}{1.002 \times 10^{-6} \, \frac{m^2}{s}} = 5.77 E4$$

(b) $N = 3.33 \frac{rev}{s}$ = 200 RPM.

Re = 9.61 E4

(c) $N = 5.00 \frac{rev}{s}$

Re = 1.44E5

Power Numbers for Rushton turbine (Fig. 2.6, $\underset{(c)}{\text{curve}}$)

$Re \geq 5.77E4$ $\quad \Phi = 5.0$

$\therefore \Phi = 5.0$ in all cases.

3/6/87

Therefore the power input is:

$$P = \frac{I \, \gamma_L \, N^3 \cdot d_I^5}{g}$$

$I = 5$

$\gamma_L = 9.79 \times 10^3 \frac{N}{m^3}$

$N = 2.0, 3.33, 5.0 \ \frac{rev}{s}$

$d_I = 0.170 \ m$

$g = 9.81 \ m/s^2$

case (a) $N = 2.00 \ \frac{rev}{s}$

$$P = \frac{5 \times 9.79 \times 10^3 \frac{N}{m^3} \times 0.170^5 \, m^5 \times 2.0^3 \frac{rev^3}{s^3} \ \frac{s^2}{m}}{9.81}$$

$$= 5.67 \ \frac{N \cdot m}{s} = 5.67 \ W$$

case (b) $N = 3.333 \ \frac{rev}{s}$

$$P = \frac{5 \times 9.79 \times 10^3 \times 0.170^5 \times 3.333^3}{9.81}$$

$$= 26.2 \ \frac{N \cdot m}{s} = 26.2 \ W$$

case (c) $N = 5.00 \ \frac{rev}{s}$

$$P = \frac{5 \times 9.79 \times 10^3 \times 0.170^5 \times 5.0^3}{9.81}$$

$$= 88.6 \ \frac{N \cdot m}{s} = 88.6 \ W$$

3/6/87			3.

2. Gassed State (20°c)

case (a) $\frac{Q_G}{N d_I^3} = \frac{3.33 \times 10^{-5} \frac{m^3}{s}}{2.0 \times 0.170^3} = 0.00339$

since N increases for case (b) & (c) then, the above number decreases

$\therefore \quad \frac{Q_G}{N d_I^3} \leq 0.00339$

$\therefore \quad \frac{P_G}{P} = 1 - 12.2 \frac{Q_G}{N d_I^3}$

case (a) $\frac{P_G}{P} = 1 - 12.2 \left(0.00339 \right) = 0.959$

$P_G = 0.959 \times 5.67 \text{ W}$

$= 5.44 \text{ W}$

$V = 0.100 \text{ m}^3 \quad \therefore \quad P_G = 54.4 \frac{W}{m^3}$

$G = \sqrt{\frac{P_G}{\mu \cdot V}}$

$= \sqrt{\frac{5.44 \frac{N \cdot m}{s}}{1.002 \times 10^{-3} \frac{N \cdot s}{m^2} \times 0.10 \text{ m}^3}}$

$= 233 \text{ s}^{-1}$

case (a) $N = 120$ RPM

$P_G = 54.4 \frac{W}{m^3}$

$G = 233 \text{ s}^{-1}$

3/6/87 | 4

case (b) $N = 3.333 \frac{rev}{s}$

$$\frac{P_G}{P} = 1 - \frac{12.2 \times 3.33 \times 10^{-5} \frac{m^3}{s}}{3.33 \times 0.170^3} = 0.975$$

$\therefore P_G = 0.975 \times 26.2 \; W$

$= 25.5 \; W$

$V = 0.100 \; m^3$ then $P_G = 255 \frac{W}{m^3}$

$$G^2 = \frac{25.5}{1.002 \times 10^{-3} \; 0.10}$$

$G = 505 \; s^{-1}$

case (b)

$N = 200$ RPM

$P_G = 255 \frac{W}{m^3}$

$G = 505 \; s^{-1}$

case (c) $N = 5 \frac{rev}{s}$

$$\frac{P_G}{P} = \frac{1 - 12.2 \times 3.33 \times 10^{-5}}{5 \times 0.170^3} = 0.983$$

$P_G = 0.983 \times 88.6 \; W$

$= 87.1 \; W$

$V = 0.100$ $\therefore$ $P_G = 871 \frac{W}{m^3}$

$$G^2 = \frac{87.1}{1.002 \times 10^{-3} \times 0.1}$$ $\therefore G = 933 \; s^{-1}$

case (c)
$N = 300$ RPM
$P_G = 871 \frac{W}{m^3}$
$G = 933 \; s^{-1}$

3. Calculate interfacial area, a

$$Re^{0.7}\left(\frac{N d_I}{v_f}\right)^{0.3}$$

$$v_f = \frac{3.33 \times 10^{-5}\ \frac{m^3}{s}}{0.196\ m^2} = 1.70 \times 10^{-4}\ \frac{m}{s}$$

case (a)

$$(5.77\ E4)^{0.7}\left(\frac{2.0 \times 0.170}{1.70 \times 10^{-4}}\right)^{0.3} = 2.11 \times 10^{4}$$

$$= 21,000$$

case (b)

$$(9.61\ E4)^{0.7}\left(\frac{3.33 \times 0.170}{1.70 \times 10^{-4}}\right)^{0.3} = 35,000$$

case (c)

$$\geq 35,000$$

- stop here.

4 Calculate RPM at which flooding of impellor is overcome.

Reference : Chemineer - Kinetics.
"Liquid Agitation, Part 6" pg. 42

$Q_G = 3.333 \times 10^{-5} \frac{m^3}{s}$ $\frac{D}{T} = \frac{D_I}{D_T} = 0.34$

$v_s = 1.70 \times 10^{-4} \frac{m}{s} \left(0.00056 \frac{ft}{s}\right)$ $V = 100\ L = 26.5$ usgal

scale of agitation = 1

$$\therefore \; Y = 1\left(0.34\right)^{1.2} = 0.27$$

from Fig. A

$$X \approx 0.01$$

$$\therefore \; X \approx \left(\frac{P}{V/1000}\right)\left(D/T\right)^{1.85}$$

$$0.01 \approx \left(\frac{P}{26.5/1000}\right)\left(0.34\right)^{1.85}$$

$$0.01 \approx \frac{1000\ P}{26.5} \cdot 0.136$$

$$P \approx \frac{0.01 \cdot 26.5}{1000 \cdot 0.136} \approx 1.95 \times 10^{-3}\ HP$$

$$\approx 1.45\ W$$

Assume $\frac{P_G}{P} = 0.95$ (conservative).

then $P \sim 1.53\ W$

$$N^3 \approx \frac{P \cdot g}{\Phi\ \gamma_L\ d_I^5} \approx \frac{1.53 \cdot 9.81}{5 \cdot 9.79E3 \cdot 0.10^5} =$$

$$\therefore \; N \approx \left(2.16\right)^{1/3} \approx 1.29\ rev/s \approx 78\ RPM.$$

APPENDIX A BIBLIOGRAPHY

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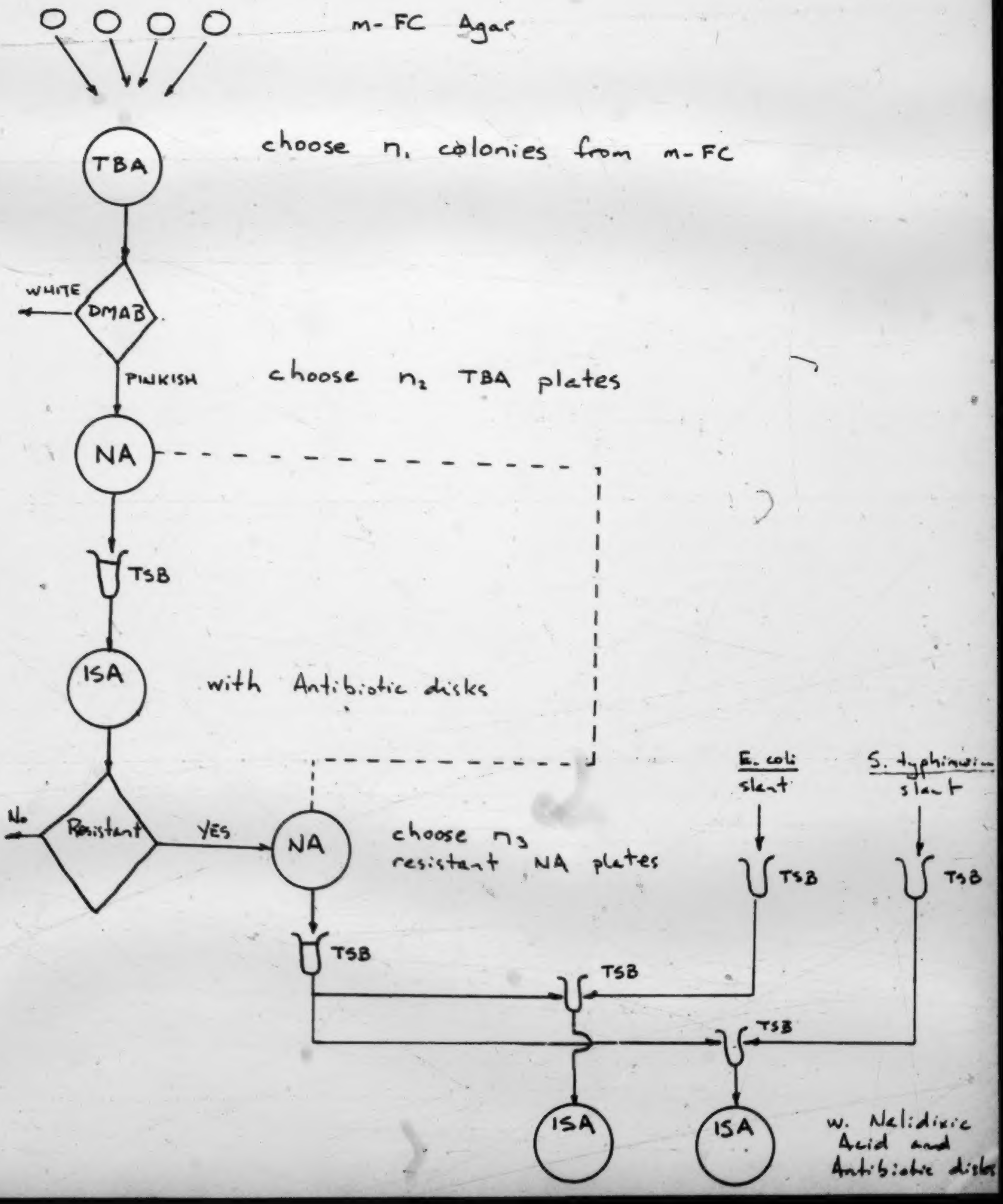
APPENDIX B: DETAILED ANTIBIOTIC-RESISTANCE TESTING PROCEDURES

1. Introduction

This laboratory manual is intended to guide the user through all phases in the process of determining the antibiotic-resistance profile of *Escherichia coli* in raw, treated and disinfected municipal wastewater.

Figure 1 illustrates the flow sheet describing how all of the following tests fit together. By following each step meticulously, consistency of the experimental results will be assured. The manual is organised in terms of the major tasks required to achieve the desired result. The first section describes the preparation of the buffered dilution water, followed by a description of the bacteriological media used in the tests including preparation and storage requirements. Other chemcial reagents are described in the subsequent section. Membrane filtration procedures occupy the next section which is followed by a complete description of antibiotic-resistance and resistance-transfer testing. The final chapter details the routine maintenance and record-keeping tasks expected for this laboratory.

Figure 1. Protocol flow chart.


m-FC Agar
TBA
choose n_1 colonies from m-FC
WHITE
DMAB
PINKISH
choose n_2 TBA plates
NA
TSB
ISA
with Antibiotic disks
No
Resistant
YES
NA
choose n_3 resistant NA plates
E. coli slant
S. typhimurium slant
TSB
TSB
TSB
TSB
TSB
ISA
ISA
w. Nalidixic Acid and Antibiotic disks

2. Dilution Water

Buffered dilution water should be prepared in accordance with Standard Methods (APHA, AWWA, WPCF, 1981). See Section 905 C, pg. 774. Final pH should be between 6 and 8. Adjust with 10% NaOH or 10% HCl. Autoclave the 90 mL dilution bottles for 15 min. at 121°C. Autoclave the 1.0 L bottles of dilution and rinse water for 30 min. at 121°C. DO NOT STORE PREPARED DILUTION WATER IN DIRECT SUNLIGHT

3. Media Preparation

3.1 m-FC Agar

Always use dehydrated media. See Standard Methods pg. 777. Prepare in accordance with manufacturer's instructions. Check that pH is 7.4 ± 0.2 after preparation. Open only one container at a time and keep tightly sealed and stored in the dark. Store prepared plates in the refrigerator for a maximum of 4 days. Bags are unnecessary. DISCARD AFTER 4 DAYS.

3.2 Tryptone Bile Agar

Dehydrated media is preferred. Prepare in accordance with manufacturer's instructions. If necessary, prepare media from components as described by Delaney *et al*. (1962). To prepare this media dissolve:

20 g Tryptone
1.5 g Bile Salt No. 3
15 g Agar

in:

1.0 L of distilled water

Check that pH is about 7.0 to 7.2. Autoclave in batches that are 1.0 L for 15 min. at 121°C. Pour the plates and store in plastic bags in the refrigerator for up to one month. DO NOT PERMIT THESE

PLATES TO DRY OUT

3.3 Nutrient Agar

Use dehydrated media. Prepare in accordance with manufacturer's instructions. If necessary prepare as follows:

> dissolve 25 g of Nutrient Broth No. 2 and 10 g of Agar in 1.0 L of distilled water.

Autoclave the same as the TBA above. Ensure pH is about 6.8 to 7.0 after autoclaving. Store in the refrigerator for up to one month. DO NOT PERMIT PLATES TO DRY OUT.

3.4 MacConkey Agar

Use dehydrated media. Prepare in accordance with the manufacturer's instructions. Store the plates in the refrigerator for up to one month in plastic sleeves. DO NOT PERMIT THESE PLATES TO DRY OUT.

3.5 Iso-sensitest Agar

Use dehydrated media and prepare according to the manufacturer's instructions. Store in the refrigerator in plastic sleeves for up to one month.

The ISA which is used for antibiotic-resistance transfer testing contains 50 µg of nalidixic acid per mL of ISA (i.e. 50 mg of nalidixic acid per L of ISA). Add 4 mL of the stock solution (12.5 mg/mL) described in the Reagent section per litre of the autoclaved ISA and stir well before pouring the plates. Store the plates as for regular ISA.

3.6 Tryptone Soya Broth

Use dehydrated media and prepare according to the manufacturer's instructions. Store in the refrigerator for up to one month. Bring to room temperature prior to use.

4. Reagents

Consult the inside front cover of Standard Methods for some standard laboratory reagent preparations.

4.1 Nalidixic Acid

Prepare a stock nalidixic acid solution containing 12.5 mg/mL by dissolving 125 mg of nalidixic acid per 10 mL of 0.2 N NaOH. Store this in the refrigerator. DO NOT AUTOCLAVE.

4.2 Sodium Hydroxide

To prepare 0.2 N NaOH from 50% w/w NaOH stock solution:

place 16.0 mL of 50% stock NaOH in a 1000 mL volumetric flask and dilute to 1000 mL mark

To prepare 1.0 N NaOH:

place 80 mL of 50% stock NaOH in a 1000 mL volumetric flask and dilute to the 1000 mL mark

4.3 Sodium Thiosulphate

Prepare stock sodium thiosulphate solution containing 1.0 mg $Na_2S_2O_3$ per mL for use in neutralizing oxidants in the distilled water. To prepare dissolve 392 mg of $Na_2S_2O_3 \cdot 5H_2O$ in 250 mL of distilled water.

Add 1.5 mL of stock solution to each L of dilution water.

4.4 p-dimethylaminobenzaldehyde

Prepare this reagent according to Vracko and Sherris (1963). p-dimethylaminobenzaldehyde (DMAB) is used for determining indole production by bacteria on TBA. To prepare this reagent, dissolve:

5.0 g DMAB

in:

100 mL of 10% aqueous HCl

Store reagent in the refrigerator.

4.5 Antibiotic Disks

These are received from the supplier in air tight dessicated packages. There are five cartridges per box. For long-term storage these are kept in the freezer. Keep Penicillin, Carbenicillin, and Chloramphenicol in the freezer all the time except when in the dispenser. NEVER PUT OPEN CARTRIDGES IN THE FREEZER OR FRIDGE. To prevent condensation and subsequent jamming of the dispenser, BRING TO ROOM TEMPERATURE BEFORE USING. Once the cartridge has been removed from its foil dessicant pack, ALWAYS STORE IN THE Oxoid Mk II DISPENSER. ALWAYS KEEP THE SCREW CAP TIGHT AND THE DESSICANT FRESH. Store the dispenser with disks in the tightly sealed container in the fridge except when in use.

4.6 Bacterial Cultures

Store the Escherichia coli K-12 strain and the Salmonella typhimurium phage type 36 strain in the refrigerator using slant tubes. Use autoclaved slant tubes and inoculate with a sterile loop. Incubate for 24 h at 35°C. Store in the refrigerator. Transfer the colonies every three months to fresh medium and test the resulting cultures for sensitivity to all antibiotics except nalidixic acid. Also test viability by growing on MacConkey Agar containing 50µg nalidixic acid per mL of agar. Use 4 mL of stock nalidixic acid solution per litre of agar after sterilization of medium. DO NOT AUTOCLAVE NALIDIXIC ACID.

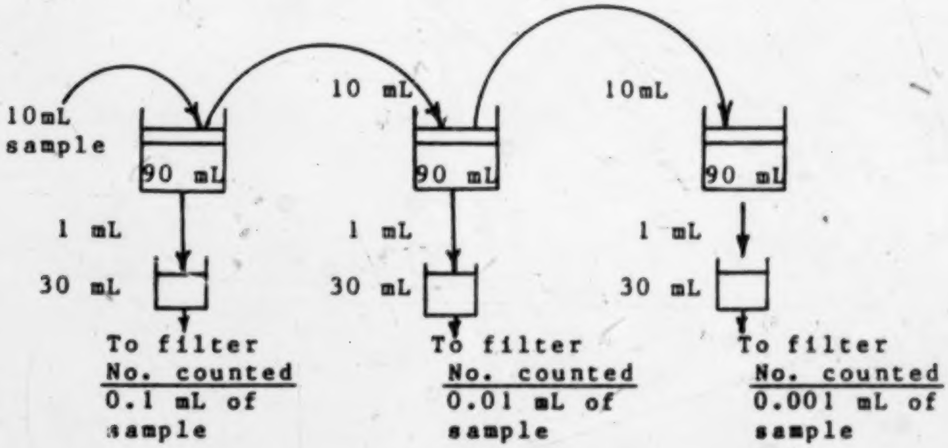
5. Fecal Coliforms Membrane Filtration

See Standard Methods 909C, pg. 814 for details of procedure.

5.1 Dilutions

It is EXTREMELY IMPORTANT that the dilutions are such that 20 to 60 colonies grow per plate. Beyond this range the technique becomes increasingly inaccurate. Also, enough plates must be filtered to ensure a minimum total population of 100 on the m-FC agar plates. Determine and record the dilutions to be used in the assay in the MEMBRANE FILTRATION DATA SHEET and use this as the basis for organizing the m-FC plates and dilution bottles.

Prepare serial dilutions using 10 mL aliquots in 90 mL dilution bottles as shown below:


10mL sample
90 mL
1 mL
30 mL
To filter
No. counted / 0.1 mL of sample
10 mL
90 mL
1 mL
30 mL
To filter
No. counted / 0.01 mL of sample
10mL
90 mL
1 mL
30 mL
To filter
No. counted / 0.001 mL of sample

Add 1 mL (or 2 mL or 5 mL as the case warrants) to a 30 mL dilution bottle in preparation for filtration. Prepare 4 plates per dilution. Usually 3 or 4 dilutions will be necessary. See

Standard Methods pg. 815 for suggested dilutions to consider. DO NOT KEEP BACTERIA SUSPENSIONS AT ROOM TEMPERATURE FOR MORE THAN 30 MINUTES before filtering.

5.2 Filtration

See Standard Methods 909C, pg.814 for details on filtration. DO NOT OPERATE VACUUM PUMPS OVER 20 psig. Ensure that vacuum bottles are empty prior to each filter run. Always start with the weakest dilution of sample in the 30 mL bottle. For example, start with the 0.0001 mL and work towards the 0.01 mL bottle. This way any carryover of bacteria to the next dilution will have a minimum effect. Incubate the filters within 30 minutes of completion of the filter.

5.3 MF Incubation

Place sufficient m-FC plates in a whirl-pak bag to ensure water does not enter the bag during the water bath incubation. Expel as much air as possible before sealing. Be sure that the bags are completely submerged and cannot come loose.

Incubate for 24 ± 2 h at 44.5 ± 0.2°C. Count the blue colonies as fecal coliforms and record the numbers in the MEMBRANE FILTRATION DATA SHEET book. Save the plates which total 100 colonies or more for further testing.

6. TBA Streaking

It is necessary to choose n fecal coliform colonies from the blue colonies on the m-FC plates according to Equation 1 (see example 1 for its application).

$$n = \frac{V^{-1}P(1-P)}{1 + N^{-1}\{V^{-1}P(1-P)-1\}} \qquad [1]$$

where:

n = required sample size

N = population size

P = true proportion of the population

p = proportion of the sample

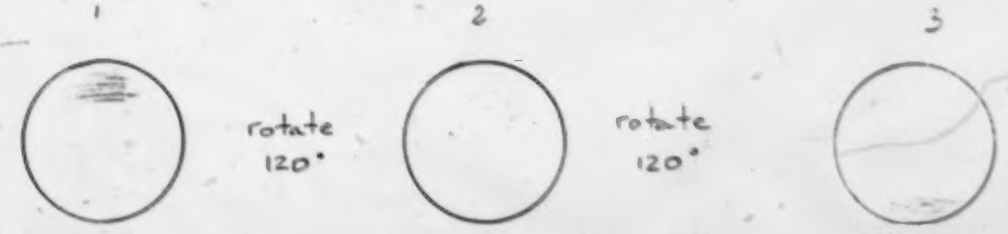
$V = \frac{d^2}{z^2}$ = desired variance of the sample proportion

z = from the normal probability distribution d = deviation from the true proportion at risk, α, of being wrong

If the population, N, is large compared to n, equation [1] reduces to:

$$n = \frac{p(1-p)}{V} \qquad [2]$$

Check with the research director for the exact number to choose since the number is a function of the expected proportion. Using a flamed sterilized wire loop, choose at random from the m-FC plates a blue colony and streak according to the picture below:



Example 1. Choice of n from m-FC isolates.

Assume E. coli population, N, is large compared to the sample size, n.

Assume that 50 % ($p = 0.50$) is the worst case for the proportion of E. coli [illegible]

Let $Pr\left(|p - P| \geq 0.15\right) = 0.10$ i.e. $\alpha = 1/10$ [illegible]

$d = 0.15$

$$n = \frac{1.65^2\,(0.5 * 0.5)}{0.15^2}$$

$$= 30$$

Therefore pick 30 E. coli for testing.

However, a certain number of blue colonies will be indole negative, therefore we need to pick an excess of blue colonies to ensure that the $n = 30$ number of E. coli remain.

Assume 75 % of the blue colonies is the worst case for E. coli, then

$$n = \frac{30}{0.75} = 40 \text{ m-FC blue colonies.}$$

Think of the plate having 3 sections: a densely populated section, a moderately populated section, and a sparsely populated section. First, heavily streak the dense section with the wire loop, then rotate the plate 120° and using another sterile wire loop streak once through the edge of the dense area and streak the moderate section of the plate. Rotate the plate another 120° and streak the final third of the plate.

Incubate the plates at 44.5°C for 20 to 24 h. Remove and place 1 drop of 5% DMAB (see reagent section above) on a well defined typical colony. Within 20 s a red-brown to pink-purple colour will develop indicating the production of indole. Record the number of coloured plates and the total number of plates tested for each sample in the laboratory notebook. KEEP THE INDOLE POSITIVE SAMPLES FOR FURTHER TESTING.

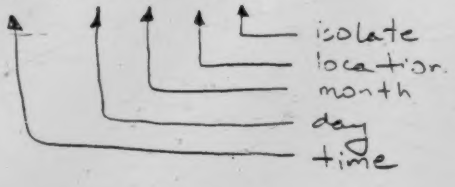
7. Antibiotic Resistance Testing

7.1 Preparing Colonies for Testing

The research director will specify the number of indole positive plates to choose for antibiotic-resistance testing. DO NOT CHOOSE A COLONY FROM THE AREA NEAR OR INCLUDING **DMAB** since it kills bacteria. Choose a typical colony and streak onto NA using a flamed sterile loop using the same technique as described above.

Next, take a sterile wire loop and pick from the most concentrated part of the NA plate. BE SURE LOOP IS COOL BEFORE PICKING. Inoculate 5 mL of sterile Tryptone Soya Broth with the wire loop. Incubate the NA plates and TSB at 35°C overnight. Code each plate if antibiotic-resistance testing is anticipated so that future reference can be made to it. Use the following example as a guide for coding:
The sample is a secondary effluent sample collected on March 11, 1986 and is the fifth plate, therefore use:

9 - 11-03-SE-5



and similarly, the twelfth plate for a raw sample collected July 18, 1986 is:

The next morning check the NA colonies for unusual morphology or other clues that would indicate that the culture was contaminated. Consult with the research director if contaminated. If the plates look fine, proceed to the next step. STORE THE CODED NUTRIENT AGAR PLATES IN THE REFRIGERATOR FOR REUSE IN FUTURE TESTING.

7.2 Antibiotic-Resistance Testing

Prepare a 10% solution of the culture growing in TSB by taking a 1.0 mL aliquot of culture and mixing it with 9 mL of sterile buffered dilution water. Ensure that the 5 mL culture is homogeneous. Using half of the 10 mL solution for each, flood two plates (there are two antibiotic disc dispensers containing different antibiotics) of regular Iso-sensitest Agar (ISA). Be sure to keep track of each sample separately. The plates should contain a uniform quantity of agar, approx. 25 mL each, which should have a uniform depth, approx. 6 mm. This is important for uniform diffusion of the antibiotics. Swish the liquid around the plate to uniformly cover the medium. Remove the excess culture with a sterile Pasteur pipette.

The Oxoid Mk II disk dispenser is used to dispense up to 6 antibiotic disks per dispenser. IT IS IMPORTANT THAT ALL ITEMS BE AT ROOM TEMPERATURE. See the instructions for the operation and maintenance of the dispenser and disks. HUMIDITY AND CONDENSATION

MUST BE PREVENTED. Keep the dispenser and cartridges in use sealed in the dessicated plastic cover except when being used. Adjust the dispenser according to the manufacturer's instructions so that each disk is applied uniformly over the surface of the (ISA). Incubate overnight at 35°C. Score the resistant isolates using the ANTIBIOTIC RESISTANCE DATA SHEET provided in the lab. Use the code described above to keep track of the colony being tested. Those organisms which grow within a 10 mm diameter of the disk may be considered resistant to the antibiotic. Those which are intermediate should be scored as sensitive. Always ensure that a Nalidixic Acid disk is present per isolate (i.e. One per pair of plates) if antibiotic-resistance transfer testing is anticipated.

7.3 Transfer Testing

All of the nalidixic acid sensitive colonies which were resistant to one or more antibiotics should be chosen for resistance-transfer testing.

Choose the resistant colony from the culture stored on nutrient agar using a long, sterile wire loop and inoculate 5 mL of TSB. Also, prepare sufficient culture in 5 mL tubes of TSB of each recipient strain of E. coli and S. typhimurium. Incubate overnight at 35°C. From a homogeneous culture of each, mix 0.1 mL of each recipient and donor in 5 mL of TSB and incubate overnight. Prepare a 10% solution of each mated culture (i.e. One E. coli mating and one Salmonella mating) and test for antibiotic resistance using the ISA plates incorporating 50 µg Nalidixic acid per mL of agar. DO NOT USE NALIDIXIC ACID DISK. Incubate for 24 h at 35°C and score for resistance as described above. Record the resistance transfers on the ANTIBIOTIC RESISTANCE DATA SHEETS as EC and/or ST indicating transfer to *E. coli* or *Salmonella typhimurium* respectively. A blank will indicate no transfer.

8. Routine Maintenance and Record Keeping

8.1 Record Keeping

Record all events in the lab in the carbon copy lab notebook on a daily basis. Cross reference the notebook to the datasheets which are kept for recording membrane filtration data and antibiotic resistance testing.

Keep duplicate records of all the datasheets and ensure that a legible carbon copy (the orange sheet) is sent to the research director (Dr. Smith).

It is important to label each batch of media as to type and date of preparation.

8.2 Maintenance

8.2.1 Incubators

Be sure to check at least daily the temperature of the incubators. Also be sure the water bath is covered and has sufficient water so that the heater element is always submerged and that any whirl-pak bags are completely submerged. The water bath should be emptied once per month and refilled to prevent the buildup of undesirable dissolved solids in the bath water.

8.2.2 Bacterial Cultures

Replenish the culture by transferring to new medium every two months. Store in the refrigerator.

8.2.3 Automatic Pipette Machine

Read maintenance instructions provided by the manufacturer and follow them carefully.

8.2.4 Disk Dispenser

Follow the directions of the manufacturer in routine cleaning of the machine.

9. References

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APPENDIX C: ANTIBIOTIC-RESISTANCE SCORE SHEETS FOR ESCHERICHIA COLI ISOLATES COLLECTED FROM RAW AND ACTIVATED SLUDGE TREATED WASTEWATER

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR
SAMPLING DATE AND TIME: 22-4-86 0900-0930 ~~[illegible]~~ & 1300-1330
ANALYSIS DATE AND TIME: 25-4-86
ANALYST: MTS
SAMPLING LOCATION: RAW INFLUENT

Isolate Reference	AMP	C	CN	~~R~~ CAR	~~FK~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks KF	~~RF~~
0900-R- 1	✓			✓		INT.	✓		✓	✓			
2	✓			✓		IN	✓		✓	✓		✓	
3						✓	✓		✓	✓			
4													STA
5													STA
6							✓						
7							✓						
8													STA
9													STA
10						✓	✓		✓	✓			
11													STA
12						✓	✓		✓	✓			
13													STA
14	✓	✓		✓	✓								
15													STA
16													STA
17						✓						✓	
18													STA
19													STA
20													STA
0900-R- 21						✓			✓	✓			
0900-R-22													STA
24													STA
25													STA
26												✓	
27					✓		✓				✓		
28						✓			✓	✓			
29													STA
0900-R-30													STA
n= 29		R%	= $\frac{13}{29}$ =	44.8%			MAR$_3$% = $\frac{9}{29}$		= 31.0%				Summary/ 0900 R
1300-R- 1						✓				✓			
2						✓							
3													STA
4							✓						
5	✓			✓		✓	✓		✓	✓			
6													STA
7						✓	✓		✓	✓		✓	
8													STA
9	✓			✓		✓			✓	✓	✓	✓	
10													STA
1300-R- 11													STA.

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR SAMPLING DATE AND TIME: 22-4-86 @ 0900-0930
ANALYSIS DATE AND TIME: 23-4-86 ANALYST: MJS 1300-1330
SAMPLING LOCATION: RAW INFLUENT

Isolate Reference	AMP	C	CN	~~R~~ CAR	~~FX~~ F	S	SF	SXT	TE	~~POS~~ OT	K	KF	Remarks
1300-R- 12													STA
13										✓			
14													STA
15	✓			✓					✓	✓			
16							✓	✓					
17													STA
18									✓	✓			
19													STA
20						✓	✓	✓	✓	✓			
21									✓	✓			
22												✓	
23													STA
24						✓	✓						
25													STA
26													STA
27	✓			✓					✓	✓			
28						✓							
29						✓	✓						
1300-R 30													STA
n= 30			R= $\frac{16}{30}$ = 53.3 %				MAR$_3$ = $\frac{6}{30}$ = 20.0 %						1300 R SUMMARY
0900-SE- 1												✓	
2	✓			✓		✓	✓		✓	✓	✓		
3													STA
4													STA
5													STA
6						✓							
7	✓			✓								✓	
8											INT	✓	
9	✓			✓					✓	✓			
10							✓	✓	✓	✓			
11	✓			✓		✓	✓		✓	✓	✓		
12						INT.			✓	✓			
13													STA
14													STA
15						✓	✓		✓	✓	✓		
16						✓	✓		✓	✓	✓		
17													STA
18													STA
19	✓			✓		✓	✓		✓	✓		✓	
20													STA
0900-SE- 21													STA.

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR SAMPLING DATE AND TIME: 22-4-86 0900-0930
ANALYSIS DATE AND TIME: 25-4-86 ANALYST: MJS 1300-1330
SAMPLING LOCATION: SECONDARY EFFLUENT

Isolate Reference	AMP	C	CH	~~R~~ CAR	~~PK~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
0900-SE-22							✓		✓	✓			
23													STA
24								✓					
25													STA
26							✓		✓	✓			
27							✓		✓	✓	✓		
28							✓						
29					✓	✓	✓		✓	✓			
30							✓						
n=	30		$R = \frac{19}{30}$	$= 63.3$ %			$MAR_3 =$	$\frac{12}{30}$	$= 40.0$ %				0900-SE SUMMARY
1300-SE-1						✓	✓						
2						✓	✓						
3													STA
4													STA
5													STA
6	✓			✓			✓	✓	✓	✓	✓		
7													STA
8						✓	✓		✓	✓			
9	✓			✓		✓	✓						
10													STA.
1300-SE-11	✓			✓		✓			✓	✓			
12											✓		
13													STA
14						✓	✓		✓	✓	✓		
15						✓							
16						✓	✓						
17	✓			✓		✓	✓		✓	✓		✓	
18	✓	✓		✓		✓	✓		✓	✓		INT	
19	✓	✓		[illegible]		✓	✓		✓	✓		INT	
20													STA
21							✓					✓	
22	✓			✓		✓	✓		✓	✓			
23													STA
24							✓		✓	✓			
25													STA
26					✓	✓							
27	✓			✓								✓	
28												✓	

n= 28 $R = \frac{19}{28}$ - 67.8 % $MAR_3 = \frac{10}{28} = 39.3$ % 1300 SE SUMMARY-

ANTIBIOTIC-RESISTANCE RECORD

PROJECT:..GOLD BAR.................... SAMPLING DATE AND TIME:..29-4-86..... @ 0900-0930 1300-1330
ANALYSIS DATE AND TIME:..2-5-86..................... ANALYST:....MJS.................
SAMPLING LOCATION:..RAW INFLUENT......................................

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~PY~~ F	S	SF	SXT	TE	TOB OT	K	KF	Remarks
0900-R-1							✓						
2													STA
3						✓							
4													STA
5													STA
6													STA
7													STA
8													STA
9													STA
10							✓						
11							✓						
12													STA
13													STA
14													STA
15													STA
16													STA
17		✓				✓	✓		✓	✓			
18													STA
19													STA
20	✓			✓									
21													STA.
0900-R-22													STA
23													STA
24													STA
25													STA
26													STA
27													STA
28	✓			✓			✓						
29													STA
30													STA
	n=30		$R = \frac{7}{30} = 23.3$ %					$MAR_3 = \frac{2}{30} = 667$ %					SUMMARY 0900-R
1300-R-1										✓			
2													STA
3					✓	✓			✓	✓			
4													STA
5													STA
6													STA
7	✓			✓		✓	✓						
8					✓				✓	✓			
9							✓						
10	✓			✓		✓	✓		✓	✓	✓	✓	

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR .... SAMPLING DATE AND TIME: 29-4-86 ...... 0900-930 1300-1330
ANALYSIS DATE AND TIME: 2-5-86 .... ANALYST: M.T.S. ....
SAMPLING LOCATION: RAW INFLUENT ....

Isolate Reference	AMP	C	CN	~~R~~ CAR	~~PY~~ F	S	SF	SXT	TE	TOB OT	K	KF	Remarks
1300-R- 11						✓			✓	✓	✓		
12						✓	✓						
13							✓		✓	✓			
14							✓						
15													STA
16								✓				✓	
17			✓		✓		✓			✓			
18													STA
19	✓			✓		✓					✓		
20													STA.
21													
22													
23													
24													
25													STA
26												✓	
27													STA
28						✓			✓	✓			
29	✓											✓	
30													STA.

n = 30 $R = \frac{16}{30} = 53.3$ % $MAR_s = \frac{9}{30} = 30.0$ % SUMMARY 1300-R

0900-SE- 1													STA
2													
3													
4													
5													STA
6	✓			✓		✓	✓	✓	✓	✓			
7	✓			✓			✓						
8						✓	✓		✓	✓			
9							✓	✓					
10													STA
11													STA
12							✓						
13						✓	✓		✓	✓			
14													STA
15													STA
16	✓			✓			✓					✓	
17													STA
18							✓						
19													STA
20													STA
21	✓			✓							✓	✓	

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR SAMPLING DATE AND TIME: 29-4-86 0900-0930 1300-1330
ANALYSIS DATE AND TIME: 2-5-86 ANALYST: MJS
SAMPLING LOCATION: SECONDARY EFF.

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~PK~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
0900-SE-22													STA
23						✓					✓		
24	✓	✓		✓		✓	✓						
25													STA
26													STA
27													STA
28	✓			✓			✓			✓		✓	
29													STA
30												✓	
	n=30		R = $\frac{13}{30}$ = 43.3 %				MAR$_3$ = $\frac{8}{30}$ = 26.7 %						Summary 0900-SE
1300-SE-1							✓	✓					
2													STA
3						✓	✓		✓	✓			
4							✓	✓	✓	✓			
5							✓	✓					
6						✓			✓	✓			
7			✓			✓			✓	✓			
8	✓			✓		✓			✓	✓	✓		
9													STA.
1300-SE-10	✓			✓	✓	✓	✓	✓	✓	✓		✓	
11													STA
12	✓			✓		✓	✓		✓	✓			
13													STA
14													
15													
16													↓
17													STA
18	✓	✓		✓			✓					✓	
19						✓	✓		✓	✓	✓		
20													STA
21	✓			✓		✓	✓		✓	✓	✓		
22	✓	✓		✓		✓	✓	✓	✓	✓	✓	[illegible]	
23	✓			✓		✓	✓						
24													STA
25													STA
26							✓						
													Summary 1300-SE
	n=26		R = $\frac{15}{26}$ = %				MAR$_3$ = $\frac{12}{26}$ = 46.2 %						

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR SAMPLING DATE AND TIME: 6-5-86 AS NOTED
ANALYSIS DATE ~~AND TIME~~: 9-5-86 ANALYST: GRF, BJW, SM, RH, SC
SAMPLING LOCATION: AS NOTED

Isolate Reference	AMP	C	CN	~~R~~ CAR	~~PT~~ F	S	SF	SXT	TE	~~DOB~~ OT	K	KF	Remarks
0900-R-6-5-1												✓	Not well defined
2												✓	"
3				—									STA
4													
5													
6													
7													STA
8	✓			✓	✓	✓			✓	✓			
9												✓	~~STA~~
10													STA
11							✓						
12	✓			✓		✓							
13							✓		✓	✓			
14											[illegible]		STA
15		✓				✓	✓		✓	✓	✓		
16							✓		✓	✓	[illegible]		
17													STA
18													
19													
20													
21													STA
0900-R-6-5-22		✓				✓	✓		✓	✓	✓		
23	✓			✓		✓			✓	✓			
24									✓	✓			
25									✓	✓			
26													STA
27													STA
28						✓			✓	✓			
29	✓					✓	✓			✓			
30													STA
	n=30		$R= \frac{15}{30} = 50.0\%$					$MAR_I = \frac{9}{30} = 30.0\%$					SUMMARY
													0900-R-6-5
1100-R-6-5-1													STA
2							✓						
3	✓					✓			✓	✓			
4							✓						
5													STA
6	✓										✓	✓	
7	✓			✓		✓	✓				✓	✓	
8							✓				✓		
9	✓				✓	✓	✓		✓	✓			
10							✓						WEAK GROWTH. ABOUT SF

ANTIBIOTIC-RESISTANCE RECORD

PROJECT:.....GOLD BAR..................... SAMPLING DATE AND TIME:....6-5-86.........
ANALYSIS DATE AND TIME:............................ ANALYST:..............................
SAMPLING LOCATION:..

Isolate Reference	AMP	C	CN	~~R~~ CAR	~~PT~~ F	S	SF	SXT	TE	TOB OT	K	KF	Remarks
1100-2-6-5- 11	✓			✓		✓			✓	✓	[illegible]	[illegible]	
12							✓	✓				✓	
13							✓						
14													STA
15	✓			✓		✓			✓	✓		✓	
16													STA
17													STA
18													STA
19	✓			✓		✓	✓		✓	✓	✓		
20													STA
21													STA
22							✓	✓				✓	
23						✓			✓	✓			
24													STA
25													
26													
27													
28													
29													
30													STA

n= 30   R= $\frac{15}{30}$ = 50.0 % $MAR_s = \frac{10}{30}$. 33.3 %

SUMMARY
1100-2-6-5

6-5-86 0900-[illegible] 1													STA
2													STA
3	✓			✓		✓							
4													STA
5							✓	✓					
6													STA
7													STA
8													STA
9	✓	✓		✓			✓						
10	✓			✓		✓	✓		✓	✓	✓		
11						✓						✓	
12						✓			✓	✓	✓		
13	✓	✓		✓		✓	✓		✓	✓			
14													STA
15													STA
16												✓	
17						✓			✓	✓	✓		
18						✓							
19													STA
20						✓	✓		✓	✓			
21													STA

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR SAMPLING DATE AND TIME: 6-5-86
ANALYSIS DATE AND TIME: ANALYST:
SAMPLING LOCATION:

Isolate Reference	AMP	C	CN	~~R~~ CAR	~~PS~~ F	S	SF	SXT	TE	~~TSB~~ OT	K	KF	Remarks
0900-6-5-SE 22	✓			✓								✓	
23													STA
24													STA
25													STA
26		✓											
27	✓			✓		✓	✓		✓	✓		✓	
28						✓	✓		✓	✓	✓		
29	✓			✓		✓	✓		✓	✓	[illegible]	✓	
30													STA
	n= 30		R= $\frac{16}{30}$ = 53.3%				MAR_3 = $\frac{11}{30}$		= 36.7 %				SUMMARY 0900-SE
1100-6-5-SE - 1	✓						✓					✓	
2													STA
3													No Growth
4	✓	✓		✓	✓	✓	✓		✓	✓	✓		
5	✓			✓	✓				✓	✓			
6						✓							
7°							✓		✓	✓			
8													STA
9											✓		
10													STA
1100-6-5-SE 11													STA
12													STA
13	✓	✓		✓		✓	✓						
14	✓			✓	✓	✓	✓		✓	✓	✓	✓	
15	✓	✓		✓			✓	✓					
16							✓						
17	✓			✓		✓			✓	✓			
18						✓			✓	✓			
19						✓			✓	✓	✓		
20													STA
21						✓			✓	✓	[illegible]		
22	✓			✓									
23													STA
24	✓			✓			✓		✓	✓			
25													STA
26							✓						
27	✓			✓		✓	✓		✓	✓	✓		
28							✓						
29													STA
30													STA -

n=29 R= $\frac{19}{29}$ = 65.5 % MAR_1 = $\frac{12}{29}$ = 41.4 % Summary 1100-SE

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR SAMPLING DATE AND TIME: 7-5-86 time as noted
ANALYSIS DATE AND TIME: 15-5-86 ANALYST: [illegible]
SAMPLING LOCATION: as noted

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
0900-R-7-5 - 1													STA
2	✓			✓			✓		✓	✓			
3							✓		✓	✓			
4							✓						
5													STA
6													STA
7						✓			✓	✓			
8													STA
9													STA
10							✓						
11							✓						
12													STA
13	✓			✓		✓	✓			✓	✓		
14						✓				✓			
15							✓						
16						✓							
17													STA
18													STA
19													STA
20	✓			✓			✓						
21													STA
0900-R-7-5- 22													STA
23							✓						
24							[illegible]						STA
25							✓		PT				[illegible]
26									✓	✓			
27													STA
28													STA
	n= 28		R= $\frac{14}{28}$ = 50.0 %					MAR_3 = $\frac{5}{28}$ = 17.9 %					Summary 0900-R-7-5
0900-SE-7-5 1							✓						
2							✓						
3	✓	✓		✓		✓	✓		✓	✓	✓		
4	✓						✓					✓	
5													STA
6													STA
7													STA
8							✓	✓	✓	✓			
9													STA
10													STA
11							✓						
12													STA

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR SAMPLING DATE AND TIME: 7-5-86
ANALYSIS DATE AND TIME: ANALYST:
SAMPLING LOCATION:

Isolate Reference	AMP	C	CN	~~R~~ CAR	~~PR~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
0900-SG-7-5 13							✓		✓	✓	✓		
14													STA
15													STA
16					✓								
17					✓	✓		✓					
18						✓	✓	✓		✓		✓	
19													STA
20	✓	✓		✓		✓	✓		✓	✓	✓		
21	~~✓~~	~~✓~~		~~✓~~			✓		✓	✓	~~✓~~		
22							✓						
23					✓		✓						
24							✓		✓	✓			
25													STA
26													STA
27	✓					✓	✓		✓	✓	✓ -	✓	
28							✓						
29							~~✓~~						STA
30							✓						
	n=30		R = $\frac{18}{30}$ = ~~[illegible]~~ 60.0 %					MAR$_3$ = $\frac{10}{30}$ = 33.3 %					summary 0900-SG-7-5
1030-R-~~[illegible]~~ 7-5 -1							✓		✓	✓			
2													No growth on TSB
3							✓						
4													STA
5						✓	✓		✓	✓			
6							✓						
7													STA.
8							✓						
9						✓	✓		✓	✓	✓		
10													STA
11													STA
12													STA
13										✓			
14													STA
15	✓	✓		✓			✓		✓	✓			
16	✓			✓		✓	✓						
17													STA
18							✓						
19	✓			✓			✓		✓	✓			
20													STA
21													STA.

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR SAMPLING DATE AND TIME: 7-5-86
ANALYSIS DATE AND TIME: ANALYST:
SAMPLING LOCATION:

Isolate Reference	AMP	C	CN	~~R~~ CAR	~~PK~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
1030-R-7-5-22							✓						
23							✓		✓	✓			
24													STA
25													STA
	n=24		$R = \frac{13}{24} = 54.2\%$				$MAR_3 = \frac{7}{24} = $ ~~[illegible]~~ 29.2 %						SUMMARY-1030-R-7-5
1030-SE-7-5-1							✓		✓	✓	✓		
2							✓						
3							✓			✓			
4													STA
5						✓	✓						
6							✓						
7													STA
8							✓						
9							✓						
10	✓			✓			✓						
11													STA
12													STA
13													STA
14													STA
15													STA
1030-SE-7-5-16							✓		✓	✓			
17							✓						
18							✓						
19	✓			✓						✓			
20													STA
21			✓		✓				✓			✓	
22						✓	✓		✓	✓			
23		✓	✓		✓								
24							✓		✓	✓			
25	✓			✓									
	n= 25			$R = \frac{17}{25} = 68.0\%$				$MAR_3 = \frac{6}{25} = 24.0\%$					

Note: Plate I.D. numbers got mixed up from 1030-SE, R-7-5-20 upto 30

However, I feel 25 to 30 are very uncertain
∴ include only upto 25.

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: Gold Bar........ SAMPLING DATE AND TIME: 7-5-86 time as noted.
ANALYSIS DATE AND TIME: 22-5-86........ ANALYST: [illegible], R.H., S.H.......
SAMPLING LOCATION: As Noted........

Isolate Reference	AMP	C	CN	~~F~~ CAR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
1230-R-7-5-1													STA
2													STA
3	✓	✓			✓	✓	✓	✓	✓	✓		✓	
4	✓				✓								
5	✓			✓								✓	
6													STA
7													STA
8					✓								
9												✓	
10					✓		✓				✓		
11													STA
12													STA
13													STA
14													STA
15													STA
16						✓	✓		✓		✓		
17													STA
18													STA
19	✓			✓				✓				✓	
20					✓								
21													STA
1230-R-7-5-22													STA
23													STA
24													STA
25													STA
26									✓	✓			
27	✓			✓		✓			✓	✓			
28													STA
	n = 28			R = $\frac{11}{28}$ = 39 %				MAR$_3$ = $\frac{6}{28}$ = 21 %					Summary 1230-R-7-5
1230-SE-7-5-1					✓								
2													STA
3							✓						
4						✓	✓		✓	✓	✓		
5													STA
6					✓	✓			✓	✓			
7													STA
8													STA
9							✓						
10													STA
11													STA
12							✓		✓	✓			

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR SAMPLING DATE AND TIME: 7-5-86
ANALYSIS DATE AND TIME: ANALYST:
SAMPLING LOCATION:

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~PF~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
1230-SE-7-513					✓								
14													STA
15													STA
16									✓	✓			
17							✓						
18							✓						
19						✓			✓	✓			
20	✓			✓					✓	✓			
21													STA
22	✓			✓		✓	✓		✓	✓			
23													STA
24													STA
25	✓			✓		✓			✓	✓			
26										✓			
27							✓	✓	✓	✓			
28													STA
29	✓			✓		✓	✓		✓	✓		✓	
30						✓	✓		✓	✓			
	n-30			R. $\frac{18}{30}$ = 60%			MAR$_3$ = $\frac{10}{30}$ = 33.3%						Summary 1230-SE-7-5
1400-R-7-5-1	✓			✓									
2					✓							--	
3	✓			✓		✓	--		✓	✓			
4													STA
5		✓					✓		✓	✓			
6					✓								
7													STA
8		✓					✓						
9													STA
10													STA
11			✓										
12		✓					✓						
13		✓					✓		✓	✓			
14													STA
15							✓		✓	✓			
16							✓			✓			
17	✓			✓							✓	✓	
18							✓		✓	✓			
19													STA
20													STA
21													STA

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: Goldbar SAMPLING DATE AND TIME: 7-5-86
ANALYSIS DATE AND TIME: ANALYST:
SAMPLING LOCATION:

Isolate Reference	AMP	C	CN	~~R~~ CAR	~~TE~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
1400-R-7-5-22										~~OF~~		~~STA~~	STA
23						✓	✓		✓	✓			
24							✓						
25													STA
26								✓					
27	✓			✓		✓	✓						
28													STA
29						✓							
30							✓						

N=30 , R= 19/30 = 63.3% , MAR_3 = 8/30 = 26.7% Summary 1400-R-7-5

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	Remarks
1400-SE-7-5-1	✓			✓		✓	✓	✓					
2										✓			
3						✓							
4													STA
5	✓			✓		✓	✓		✓	✓			
6	✓			✓		✓	✓		✓	✓			
7							✓						
8							~~✓~~		~~✓~~	~~✓~~			STA
9							✓		✓.	✓			~~[illegible]~~
10													STA
1400-SE-7-5-11							✓						
12								✓					
13												~~STA~~	STA
14							✓						
15							✓						
16							✓			✓			
17													STA
18	✓			✓		✓			✓	✓			
19							✓						
20	✓			✓		✓	✓		✓	✓			
21	✓			✓		✓	✓						
22							✓						
23													STA
24							✓						
25							✓						
26							✓						
27													STA
28						✓			✓	✓	✓		
29						✓	✓		✓	✓	✓		
30		✓					✓		✓	✓			

n=30, R = 23/30 = 76.7% , MAR_3 = 10/30 = 33.3% Summary 1400-SE-7-5

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR .... SAMPLING DATE AND TIME: 13-5-86 ..... time as noted
ANALYSIS DATE AND TIME: 27-5-86 .... ANALYST: R.T.H, R.H, J.A. ....
SAMPLING LOCATION: As Noted ....

Isolate Reference	AMP	C	CN	~~R~~ CAR	~~PK~~ F	S	SF	SXT	TB	TOB. OT	K	KF	Remarks
0900-R-13-5-1								✓					
2	✓			✓		✓	✓		✓	✓			
3													STA
4	✓												
5							✓						
6	✓			✓		✓	✓		✓	✓			
7	✓						✓	✓					
8								✓				✓	
9								✓	✓	✓			
10													STA
11	✓												
12							✓						
13								✓	✓	✓		✓	
14	✓											✓	
15							✓					✓	
16	✓							✓				✓	
17								~~✓~~				~~✓~~	STA
18													~~STA~~ STA
19						✓	✓						
20							✓						
21					✓		✓		✓	✓		✓	
0900-R-13-5-22							✓				✓	✓	
23							✓					✓	
24													STA
25							✓					✓	
26	✓			✓		✓	✓		✓	✓			
27				✓		✓	✓		✓	✓		✓	
28	✓			✓	✓	✓	✓		✓	✓		✓	
29							✓						
30													STA
summary 0900-R-13-S	n = 30, R = 24/30 = 80%, MAR_s = 10/30 = 33.3%												
0900-E-13-5-1							✓						
2	✓			✓									
3													STA
4						✓	✓		✓	✓			
5												✓	
6							✓						
7							✓						
8													STA
9													STA
10	✓			✓									

ANTIBIOTIC-RESISTANCE RECORD

PROJECT:...Goldbar........................ SAMPLING DATE AND TIME:....................
ANALYSIS DATE AND TIME:......................... ANALYST:..........................
SAMPLING LOCATION:..

Isolate Reference	AMP	C	CN	~~R~~ CAR	~~RX~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
0900-SE-13-5-11							✓		✓	✓			
12													STA
13	✓	✓		✓		✓	✓		✓	✓	✓		
14									✓	✓			
15													STA
16													STA
17						✓	✓		✓	✓	✓		
18	✓			✓			✓		✓	✓		✓	
19													STA
20	✓			✓			✓						
21							✓						
22													STA
23	✓	✓		✓		✓	✓		✓	✓			
24													STA
25	✓	✓		✓		✓	✓						
26	✓			✓		✓	✓		✓	✓			
27	✓	✓		✓		✓	✓		✓	✓			
28		✓		✓			✓					✓	
29							✓						
30						✓						✓	

summary 0910-SE-13-5 n=30. R=21/30=70%. MAR_3 = 11/30 = 36.7%.

1100-R-13-5-1							✓						
2							✓						
3	✓								✓	✓			
4	✓						✓						
5							✓						
6	✓			✓			✓						
7	✓			✓			✓	✓					
8								✓				✓	
9						✓	✓	✓					
10								✓					
11				✓		✓	✓	✓	✓	✓			
12	✓			✓			✓	✓					
13	✓	✓		✓			✓	✓					
14						✓		✓					
15						✓		✓	✓	✓			
16	✓			✓			✓	✓				✓	
17	✓			✓	✓		✓	✓				✓	
18								✓					
19							✓						
20							✓	✓					
21								✓					

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: Goldbar SAMPLING DATE AND TIME:
ANALYSIS DATE AND TIME: ANALYST:
SAMPLING LOCATION:

Isolate Reference	AMP	C	CN	CAR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
1100-R-13-5-22							✓						
23							✓						
24							✓						
25		✓					✓				✓		
26													STA
27						✓	✓		✓	✓		✓	
28		✓				✓	✓		✓	✓			
29			✓				✓						
30							✓					✓	
Summary:													
1100-13-5-R			n=30		R: $\frac{29}{30}$ = 96.7%				MAR = $\frac{12}{30}$ = 40%				
1100-SE-13-5-1													STA
2		✓				✓		✓					
3	✓			✓		✓	✓		✓	✓			
4													STA
5	✓			✓		✓			✓	✓			
6												✓	
7						✓	✓						
8	✓			✓		✓			✓	✓			
9	✓			✓			✓						
1100-13-5-SE-10											~~STA~~		STA
11	✓			✓		✓			✓	✓			
12													STA
13								✓			✓		
14													STA
15	✓	✓		✓		✓	✓	✓	✓	✓	✓		
16	✓			✓			✓		✓	✓			
17		✓					✓						
18	✓	✓		✓		✓	✓				✓	✓	
19													STA
20						✓							
21							✓						
22	✓			✓			✓	✓	✓	✓	✓		
23	✓			✓		✓	✓	✓					
24							✓	✓	✓	✓		✓	
25	✓			✓			✓	✓				✓	
26	✓			✓				✓	✓	✓			
27													STA
28	✓			✓				✓				✓	
29	✓							✓				✓	
30	✓			✓			✓	✓	✓	✓			

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: Goldbar SAMPLING DATE AND TIME:
ANALYSIS DATE AND TIME: ANALYST:
SAMPLING LOCATION:

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~FT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
Summary:		1100-13-5-SE								ε			
			n = 30			R = 23/30	= 76.7%	MAR₃		= 17/30	= 56.7%		
R-1300-13-5-1	✓												
2										✓			
3													~~STA~~ STA
4									✓	✓			
5												✓	
6													STA
7							✓						
8												✓	
9	✓			✓			✓		✓	✓			
10													STA
11	✓			✓	✓	✓	✓		✓	✓	✓		
12						~~✓~~	~~✓~~		~~✓~~	✓			
13	✓			✓		✓	✓		✓	✓	✓	✓	
14						~~✓~~	~~✓~~		~~✓~~	~~✓~~		~~✓~~	STA
15													STA
16					✓		✓						
17							✓						
R-1300-13-5-18													STA
19													STA
20						✓	✓		✓	✓			
21						✓							
22						✓						✓	
23													STA
24						✓		✓	✓	✓			
25						✓					✓	✓	
26							✓						
27						✓		✓	✓	✓			
28													STA
29						✓							
30													STA
Summary R-1300-13-5	n = 30		R = 20/30 = 66.7%				, MAR₃ =			7/30 = 23.3%			
SE-1300-13-5-1							✓						
2							✓						
3							✓						
4									✓				
5					✓	✓	✓	✓	✓	✓	✓		
6							✓			✓			

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: Goldbar .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: ....

Isolate Reference	AMP	C	CN	~~R~~	~~PY~~	S	SF	SXT	TE	~~TOB~~		Remarks	
				CAR	F					OT	K	LF	
1300-SE-13-5-7						✓							
8	✓			✓		✓			✓	✓			
9							✓					✓	
10					✓								
11													STA
12						✓							
13	✓	✓			✓		✓					✓	
14					✓	✓			✓	✓			
15							✓		✓	✓			
16									✓	✓			
17	✓	✓		✓			✓	✓	✓	✓			
18	✓			✓		✓	✓		✓	✓			
19									✓	✓			
20	✓			✓		✓	✓					✓	
21						✓			✓	✓		✓	
22												✓	
23			✓				✓					✓	
24												✓	
25	✓			✓		✓	✓					✓	
26										✓			
27							✓						
1300-SE-13-5-28	✓			✓		✓	✓		✓	✓		✓	
29						✓		✓	✓	✓			
30						✓	✓		✓	✓			
	n = 30, R = 29/30 = 96.7%, MAR_3 = 13/30 = 43.3%												
R-1430-13-5-1										✓			
2												✓	
3													STA
4					✓								
5						✓	✓		✓	✓	✓		
6													STA
7	✓			✓		✓					✓		
8		✓					✓			✓			
9							✓						
10									✓	✓			
11			✓				✓						
12	✓			✓		✓	✓		✓	✓	✓	✓	
13	✓			✓	✓	✓	✓		✓	✓			
14													STA
15							✓						
16	✓			✓			✓					✓	

ANTIBIOTIC-RESISTANCE RECORD

PROJECT:...Goldbar.................... SAMPLING DATE AND TIME:..................
ANALYSIS DATE AND TIME:.............................. ANALYST:.......................
SAMPLING LOCATION:..

Isolate Reference	AMP	C	CN	~~R~~ CAR	~~PY~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
R-1430-13-5-17						✓	✓	✓	✓	✓	✓		
18							✓	✓	✓	✓			
19													STA
20													STA
21					✓		✓				✓	✓	
22					✓	✓	✓		✓	✓	✓		
23					✓		✓						
24							✓				✓		
25						✓							
26							✓						
~~27~~													
~~28~~													
~~29~~													
~~30~~													
	n= 26 , R = 21/ 26 = 80.8% . MAR$_3$ = 10/26 = 38.5%												
SE-1430-13-5-1							✓						
2													STA
3		✓				✓							
4							✓						
5													STA
1430-SE-13-5-6						✓	✓						
7			✓			✓			✓	✓			
8					✓		✓			✓			
9						✓	✓		✓	✓			
10					✓	✓	✓		✓	✓			
11	✓											✓	
12	✓			✓			✓		✓	✓		✓	
13													STA
14	✓			✓			✓						
15							✓						
16									✓	✓	✓	✓	
17					✓	✓					✓		
18													STA
19							✓			✓			
20									✓	✓			
21							✓						
22						✓	✓		✓	✓			
23						✓	✓						
24							✓				✓	✓	
25							✓						
26							✓						
1430-SE-13-5-27							✓					✓	
28						✓			✓	✓			
29	✓			✓			✓		✓	✓			
30													STA
	n=30 R= 25/30 = 83.3 % , MAR$_3$ = 12 /30 = 40 %												

APPENDIX D: ANTIBIOTIC-RESISTANCE SCORE SHEETS FOR *ESCHERICHIA COLI* ISOLATES COLLECTED FROM ACTIVATED SLUDGE TREATED WASTEWATER BEFORE AND AFTER OZONE DISINFECTION

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR SAMPLING DATE AND TIME: 12-06-86 time as noted
ANALYSIS DATE AND TIME: 12-06-86 ANALYST: BJY, RH
SAMPLING LOCATION: As Noted - OZONATION EXPERIMENTS

Isolate Reference	AMP	C	CM	~~R~~ CAR	~~TK~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks KF	
1200-SE-12-06-1						✓			✓	✓			
2							✓					✓	
3	✓			✓		✓	✓		✓	✓			
4							✓		✓	✓		✓	
5							✓		✓	✓			
6							✓					✓	
7							✓					✓	
8													STA
9							✓					✓	
10							✓		✓		✓		
11	✓											✓	
12							✓		✓	✓	✓	✓	
13							✓						
14	✓			✓			✓	✓		✓		✓	
15													STA
16								✓	✓				
17										✓		✓	
18							✓						
19													STA
20							✓						
21													STA
1200-SE-12-06-22							✓		[illegible]			✓	
23									✓	✓			
24													STA
25													STA
26							✓						
27	✓			✓			✓						
28													STA
29	✓			✓		✓			✓	✓			
30							✓					✓	

n=30 R= 23/30 = [illegible] 77% MAR$_2$ = 19/30 = 63% Summary 1200-SE-12-06
MAR$_3$ = 8/30 = 27%
MAR$_4$ = 5/30 = 17%
MAR$_5$ = 4/30 = 13%
MAR$_6$ = 2/30 = 6.7%

Isolate Reference	AMP	C	CM	CAR	F	S	SF	SXT	TE	OT	K	KF	Remarks
1200-OE-12-06	DEAD (NA Plate)												
2													STA
3	✓			✓		✓						✓	
4			✓			✓.	✓		✓	✓			
5		✓							✓	✓	✓		
6							✓		✓				
7													STA

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: 3

Isolate Reference	AMP	C	CN	~~T~~ CAR	~~PR~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks KF	
1200-BE-12-06-8												✓	
9							✓						
10									✓	✓			
11							✓						
12					✓								
13													STA
14									✓	✓			
15	✓			✓		✓							
16							✓					✓	
17						✓	✓		✓	✓		✓	
18	✓			✓		✓	✓					✓	
19							✓						
20						✓			✓	✓			
21	✓			✓		✓	✓					✓	
22						✓	✓		✓	✓		✓	
23							✓					✓	
24													STA
25						✓	✓			✓		✓	
26						✓	✓		✓	✓	✓	✓	
27							✓					✓	
28													STA
1200-02-12-06-29													STA
30													STA
	n = 29		R = 22/29 = 76%				MAR_2 = 17/29 =			59%			Summary
							MAR_3 = 11/29 =			38%			1200-02-12-06
							MAR_4 = 9/29 =			31%			Dose
							MAR_5 = 5/29 =			17%			4.86 mg/L
							MAR_6 = 1/29 =			3.4%			
1300-SE-12-06-1						✓	✓					✓	
2							✓					✓	
3	✓	✓			✓							✓	
4	✓			✓			✓					✓	
5							✓						
6												✓	
7							✓					✓	
8							✓						
9							✓					✓	
10							✓						
11	✓			✓			✓					✓	
12													STA
13							✓		✓	✓		✓	
14		✓			✓							✓	

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: 5

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~BY~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks KF	
1300-SE-12-06-15							✓					✓	
16					✓				✓	✓	✓		
17													STA
18							✓		✓	✓		✓	
19													STA
20							✓					✓	
21							✓					✓	
22							✓						
23													STA
24	✓	✓		✓		✓	✓	✓	✓	✓	✓	✓	
25	✓	✓		✓		✓	✓	✓	✓	✓	✓		
26		✓				✓	✓					✓	
27	✓												
28							✓						
29													STA
30	✓			✓		✓			✓	✓	✓		

n = 30 R = 25/30 = 83% MAR_2 = 18/30 = 60% Summary
MAR_3 = 12/30 = 40% 1300-SE-12-06
MAR_4 = 10/30 = 33%
MAR_5 = 3/30 = 10%

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	Remarks
1300-OZ-12-06-1							✓						
2									✓	✓		✓	
3													STA
4	✓			✓		✓	✓		✓	✓			
5													STA
6	✓			✓			✓		✓	✓			
7	✓			✓		✓	✓	✓	✓	✓		✓	
8													STA
9													STA
10													STA
11													STA
12													STA
13													STA
14													STA
15	✓			✓								✓	
16													STA
17	✓			✓		✓			✓	✓	✓	✓	
18													STA
19						✓			✓	✓			
20	✓			✓		✓			✓	✓	✓		
21													STA

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: 4

Isolate Reference	AMP	C	CN	~~K~~ CLR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks KF	
1300-02-12-06-22													STA
23													STA
24													STA
25													STA
26									✓	✓			
27													STA
28													STA
29													STA
30													STA

n = 30 R = 10/30 = 33% MAR_2 = 9/30 = 30 % Summary
MAR_3 = 8/30 = 27 % 1300-02-12-α
MAR_4 = 5/30 = 17 % Dose
MAR_5 = 5/30 = 17 % 2.37 mg/L O_3
MAR_6 = 4/30 = 13 %

Isolate Reference	AMP	C	CN	~~K~~ CLR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks KF	
1115-SE-18-06-1						✓	✓		✓	✓	✓		
2													STA
3							✓						
4													STA
5							✓						
6							✓						
7							✓		✓	✓			
8							✓						
9													STA
10							✓		✓	✓	✓		
11													STA
12							✓						
13													STA
14													STA
15						~~✓~~	✓						STA
16						✓	✓	~~✓~~					
17							✓	✓					
18							✓		✓	✓			
19	✓			✓		✓							
20	✓			✓		✓	✓		✓	✓			
21							✓						

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: ....

②

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks KF	
1115-SE-18-06-22	✓			✓		✓	✓						
23													STA
24						✓	✓		✓	✓			
25													STA
26							✓		✓	✓			
27	✓	✓		✓			✓						
28													STA
29							✓		✓	✓			
30									✓	✓			
	n = 30		R = 20/30 = 66.7%				MAR_2 =		14/30 = 46.7%			Summary	
							MAR_3 =		11/30 = 36.7 %			1115-SE-18-06	
							MAR_4 =		6/30 = 20.0 %				
							MAR_5 =		2/30 = 6.67 %				
							MAR_6 =		1/30 = 3.33 %				
1115-07-18-06-1							✓	✓					
2							✓	✓					
3							✓						
4	✓			✓		✓	✓		✓	✓			
5							✓						
6						✓	✓						
7	✓	✓		✓			✓		✓	✓			
1115 07-18-06-8	✓			✓					✓	✓	✓		
9		✓			✓	✓	✓		✓	✓	✓	✓	
10	✓			✓			✓		✓	✓			
11						✓			✓	✓			
12													STA
13													STA
14													STA
15													STA
16													STA
17							✓						
18	✓			✓		✓			✓	✓			
19													STA
20													STA
21		✓		✓	✓		✓		✓	✓			
22				✓	✓								
23													STA
24							✓		✓	✓			
25							✓		✓	✓			
26													STA
27	✓	✓		✓	✓	✓	✓		✓	✓	✓		
28							✓						

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: ....

④

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks	
												KF	
1115-~~OZ~~-18-06-29												STA	STA
30												STA	STA
	n=30		R=19/30=63.3%			MAR$_2$ = 15/30 = 50.0%					Summary	1115-~~SE~~-18-06 OZ	
						MAR$_3$ = ~~11/30~~ = 36.7%							
						MAR$_4$ = 8/30 = 26.7%							
						MAR$_5$ = 8/30 = 26.7%							
						MAR$_6$ = 5/30 = 16.7%							
1215-SE-17-06-1			✓				✓						
2													STA
3								✓	✓	✓			
4					✓								
5							✓						
6							✓		✓	✓			
7	✓			✓			✓	✓					
8						✓							
9	✓	✓		✓			✓		✓	✓	✓		
10	✓			✓		✓			✓	✓			
11	✓			✓		✓	✓		✓	✓			
12	✓	✓		✓		✓	✓		✓	✓	✓		
13							✓		✓	✓		✓	
14							✓		✓	✓		✓	
1215-SE-17-06-15													STA
16	✓			✓		✓	✓		✓	✓	✓		
17													STA
18							✓						
19							✓					✓	
20													STA
21					✓	✓	✓		✓	✓			
22					✓		✓						
23					✓		✓						
24									✓	✓			
25									✓	✓			
26	✓		✓	✓		✓	✓		✓	✓	✓		
27	✓			✓		✓			✓	✓			
28	✓			✓			✓					✓	
29	✓	✓		✓				✓			✓	✓	
30	✓	✓		✓			✓	✓				✓	
	n=30		R=26/30=86.7%			MAR$_2$ = 22/30 = 73.3%					Summary	1215-SE-17-06	
						MAR$_3$ = 16/30 = 53.3%							
						MAR$_4$ = 14/30 = 46.67%							
						MAR$_5$ = 10/30 = 33.3%							

MAR$_6$ = 7/30 = 23.3%

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR ....SAMPLING DATE AND TIME: ....
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: ....

⑦

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~P~~ F	S	SF	SXT	TE	~~DOB~~ OT	K	Remarks KF	
1215-OZ-17-06-1									✓	✓	✓	✓	
2	✓	✓		✓									
3							✓		✓	✓			
4									✓	✓	✓		
5							✓		✓	✓			
6						✓	✓		~~✓~~	✓			
7						✓	✓			✓			
8						✓	✓			✓	✓		
9						✓	✓		✓		✓	✓	
10							✓						~~STA~~
11							~~✓~~						STA
12							✓						~~STA~~
13							✓						~~STA~~
14													STA
15							✓						
16							✓		✓	✓			
17													STA
18													STA
19							✓						
20						✓	✓						
21													STA
1215-OZ-17-06-22													STA
23							✓						
24													STA
25													STA
26							✓						
27							✓						
28													STA
29													STA
30	✓			✓		✓	✓	✓	✓	✓		✓	

n = 30 R = 20/30 = 66.7% MAR_2 = 12/30 = 40%
MAR_3 = 11/30 = 36.7%
MAR_4 = 4/30 = 13.3%
MAR_5 = 2/30 = 6.66%
MAR_6 = 1/30 = 3.33%

Summary
1215-OZ-17-06

1330-SE-17-06-1							✓						
2							✓						
3									✓	✓			
4							✓						
5							✓						~~STA~~
6													STA
7													STA

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLD BAR SAMPLING DATE AND TIME:
ANALYSIS DATE AND TIME: ANALYST:
SAMPLING LOCATION:

⑨

Isolate Reference	AMP	C	CN	~~F~~ CAR	~~FT~~ F	S	SF	SXT	TE	~~DOS~~ OT	KF	Remarks KF	
1330-SE-17-06-8							✓						
9							✓						
10							✓						
11							✓						
12							✓		✓	✓			
13	✓			✓		✓					✓		
14							✓						
15	✓			✓									
16							✓						
17							✓						
18													STA
19													STA
20													STA
21													STA
22						✓	✓		✓	✓			
23													STA
24													STA
25													STA
26									✓	✓			
27													STA
28						✓			✓	✓			
1330-SE-17-06-29													STA
30													STA

Summary 1330-SE-17-06

n = 30 R = ~~22~~ ~~20~~ 18/30 = ~~73.3%~~ ~~66.7%~~ 60.0%

MAR$_2$ = 7/30 = 23.3 %
MAR$_3$ = 4/30 = 13.3 %
MAR$_4$ = 2/30 = 6.67 %
MAR$_5$ = 0/30 = 0 %
MAR$_6$ = 0/30 = 0 %

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	KF	KF	
1330-0Z-17-06-1											✓		
2													STA
3													STA
4						✓	✓		✓	✓			
5													STA
6													STA
7					✓								
8					✓	✓			✓	✓	✓		
9					✓								
10													STA
11						✓	✓		✓	✓			
12													STA
13													STA

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR .... SAMPLING DATE AND TIME: 11
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: ....

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
1330-07-17-06-14							✓						
15							✓						
16						✓	✓		✓	✓			
17													STA
18					✓	✓	✓	✓	✓	✓			
19						✓	✓				✓		
20						✓							
21													STA
22						✓			✓	✓			
23						✓							STA
24						✓					✓		
25						✓	✓						
26						✓	✓		✓	✓			
27						✓					✓		
28					✓	✓	✓						
29													STA
30	✓			✓									

Summary 1330-02-17-06 n = 30, R = 19/30 = 63.3 %

MAR$_2$ = 13/30 = 43.3 % MAR$_5$ = 2/30 = 6.67 %

MAR$_3$ = 9/30 = 30.0 % MAR$_6$ = 1/30 = 3.33 %

MAR$_4$ = 6/30 = 20.0 %

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	Remarks
1015-02-29-06-1													STA
2							✓		✓	✓			
3						✓			✓	✓	✓		
4	✓			✓		✓	✓		✓	✓	✓		
5													STA
6							✓						
7							✓						
8							✓		✓	✓			
9	✓			✓		✓	✓		✓	✓			
10							✓						
11							✓						
12						✓	✓		✓	✓	✓		
13							✓		✓	✓			
14							✓						
15							✓						
16							✓						
17							✓		✓	✓			
18							✓						
19	✓			✓			✓		✓	✓			
20							✓						
21	✓			✓		✓	✓		✓	✓	✓	✓	

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: .... ②

Isolate Reference	AMP	C	CN	~~P~~	~~PT~~	S	SF	SXT	TE	~~TOS~~		Remarks	
				CAR	F					CT	K	KF	
1015-OE-24-06-22							✓						
23	✓			✓		✓			✓	✓			
24							✓						
25							✓						
26													STA
27							✓						
28							✓						
29							✓						
30							✓						

n = 30 R = 27/30 = 90.0% MAR$_2$ = 11/30 = 36.7%
MAR$_3$ = 11/30 = 36.7%
MAR$_4$ = 7/30 = 23.3%
MAR$_5$ = 5/30 = 16.7%
MAR$_6$ = 3/30 = 10.0%

Summary
1015-OE-24-06

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	CT	K	KF	Remarks
1015-SE-24-06-1							✓						
2		✓					✓		✓	✓	✓		
3	✓	✓		✓		✓	✓	✓	✓	✓	✓		
4							✓						
5							✓						
6													
7							✓						STA
1015-SE-24-06-8							✓						
9							✓						
10							✓						
11	✓			✓		✓	✓	✓				✓	
12	✓			✓		✓	✓		✓	✓	✓		
13							✓						
14	✓	✓		✓		✓	✓		✓	✓			
15							✓						
16	[illegible]						✓		✓	✓		[illegible]	
17	✓						✓					✓	
18							✓						
19						[illegible]	✓		✓	✓			
20							✓		✓	✓	✓		
21							✓						
22							✓						
23							✓		✓	✓			
24							✓						
25							✓					✓	
26							✓						
27							✓		✓	✓			

n = 27 R = 26/27 = 96.3% MAR$_2$ = 12/27 = 44.4% MAR$_4$ = 6/27 = 22.2% MAR$_6$ = 4/27
M.AR$_3$ = 11/27 = 40.7% MAR$_5$ = 5/27 = 18.5% = 14.8

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR SAMPLING DATE AND TIME:
ANALYSIS DATE AND TIME: ANALYST:
SAMPLING LOCATION: ④

Isolate Reference	AMP	C	CN	~~F~~ CAR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks KF	
1445-08-23-06-1							✓						
2							✓						
3							✓						
4							✓		✓	✓			
5						✓	✓		✓	✓			
6							✓		✓	✓			
7	✓			✓			✓					✓	
8						✓	✓						
9													STA
10							✓						
11							✓						
12							✓						
13	✓			✓			✓						
14							✓						
15							✓						
16							✓		✓	✓			
17	✓			✓		✓	✓		✓	✓			
18							✓		✓	✓			
19							✓						
20							✓		✓	✓			
21													STA
1445-07-23-06-22						✓	✓						
23							✓				✓		
24							✓						
25							✓						
26							✓						
27							✓						
28							✓						
29							✓						
30							✓						

n = 30

R = 28/30 = 93.3% $\text{MAR}_2 = 12/30 = 40.0\%$

$\text{MAR}_3 = 9/30 = 30.0\%$

$\text{MAR}_4 = 3/30 = 10.0\%$

$\text{MAR}_5 = 1/30 = 3.33\%$

$\text{MAR}_6 = 1/30 = 3.33\%$

summary
1445-07-23-86

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	
1445-[illegible]-23-06 1							✓		✓	✓			
2							✓						
3	✓			✓		✓	✓	✓	✓	✓			
4							✓		✓	✓			
5						✓	✓		✓	✓			
6							✓						
7	✓			✓		✓	✓						

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR SAMPLING DATE AND TIME:
ANALYSIS DATE AND TIME: ANALYST:
SAMPLING LOCATION:

⑥

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~PT~~ F	S	SF	SXT	TE ✓	~~POB~~ OT	K	Remarks KF	
1445-SE-23-06-8							✓						
9							✓						
10							✓		✓	✓			
11							✓	✓					
12							✓	✓					
13						✓	✓		✓	✓			
14							✓						
15							✓						
16						✓	✓		✓	✓			
17													STA
18							✓						
19							✓						
20	✓			✓		✓	✓				✓		
21							✓						
22											✓		
23			✓				✓	✓			✓		
24						✓	✓		✓	✓	✓		
25							✓						
26							✓		✓	✓			
27							✓		✓	✓			
28						✓	✓		✓	✓			
1445-SE-23-06-29	✓			✓		✓	✓		✓	✓			
30							✓						
	n=30		R = 29/30 = 96.7%			$MAR_2 = 17/30 =$			56.7%			Summary	
						$MAR_3 = 15/30 =$			50.0%			1445-SE-23-06	
						$MAR_4 = 10/30 =$			33.3%				
						$MAR_5 = 3/30 =$			10.0%				
						$MAR_6 = 2/30 =$			6.67%				
915-OE-24-06-1							✓						
2							✓						
3						✓	✓		✓	✓			
4							✓						
5						✓	✓		✓	✓			
6							✓					✓	
7							✓						
8							✓		✓	✓		✓	
9							✓						
10							✓						
11							✓						
12							✓						
13						✓	✓		✓	✓			
~~14~~					dead testtube of TSB								

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: .... (F)

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks KF	
915-OZ-24-06-15	✓			✓		✓	✓		✓	✓			
16							✓						
17							✓						
18							✓						
19							✓						
20	✓			✓		✓	✓		✓	✓			
21	✓		✓	✓		✓	✓		✓	✓	✓		
22						✓	✓			✓			
23						✓	✓		✓	✓	✓		
24							✓						
25	✓	✓		✓		✓	✓		✓	✓			
26							✓		✓	✓			
27													JVA
28							✓						
29							✓						
30							✓						
915-SE-24-06-1							✓						
2							✓						
3							✓						
4							✓		✓	✓	✓		
5							✓						
6							✓						
7	✓			✓		✓	✓		✓	✓			
8							✓						
9	✓			✓		✓	✓	✓					
10							✓						
11	✓			✓		✓	✓	✓	✓	✓			
12							✓						
13						✓	✓		✓	✓			
14							✓		✓	✓			
15							✓						
16			✓				✓						
17							✓						
18							✓		✓	✓			
19							✓						
20							✓						
21			✓				✓		✓	✓			
915-SE-24-06-22			✓		✓		✓			✓			
23					✓		✓	✓					
24							✓						

n = 24

R = 24/24 - 100.0%

MAR_2 = 11/24 = 45.8% Summary

MAR_3 = 10/24 = 41.7% 915-SE-24-06

MAR_4 = 7/24 = 29.2%

MAR_5 = 3/24 = 12.5%

MAR_6 = 2/24 = 8.33%

APPENDIX E: ANTIBIOTIC-RESISTANCE SCORE SHEETS FOR *ESCHERICHIA COLI* ISOLATES COLLECTED FROM ACTIVATED SLUDGE TREATED WASTEWATER FOR THE OZONE DOSE-RESPONSE EXPERIMENTS

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR SAMPLING DATE AND TIME: 15-07-86 @ 1300
ANALYSIS DATE AND TIME: 19-07-86 ANALYST: BTH
SAMPLING LOCATION: OZONATOR - [illegible] ①

Isolate Reference	AMP	C	CN	~~F~~ CAR	~~PY~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks KF	
1300-15-07-0.01													STA
2													STA
3							✓		✓	✓			
4							✓						
5							✓						
6							✓						
7							✓						
8													STA
9													STA
10	✓			✓			✓		✓	✓			
11													STA
12							~~✓~~						STA
13							~~✓~~						STA
14							✓						
15													STA
16													STA
17													STA
18	✓			✓		✓	~~✓~~		✓	✓	✓		
19													STA
20						✓	✓						
21							✓						
1300-15-07-00-22													STA
23													STA
24													STA
25	✓			✓			✓		✓	✓	✓		
26													STA
27	✓			✓		✓	~~✓~~				✓		
28							~~✓~~		✓	✓			
29							~~✓~~				✓		
30							~~✓~~						STA

n = 30 R = ~~[illegible]~~ 14/30 = ~~[illegible]~~ 46.7% MAR$_2$ = ~~[illegible]~~ 7/30 = ~~[illegible]~~ 23.3 % Summary

MAR$_3$ = 5 ~~6~~/30 = ~~20.0~~ 16.6 % 1300-15-07-0.0

MAR$_4$ = 4/30 = 13.3%

MAR$_5$ = 3 ~~4~~/30 = ~~13.3~~ 10.0 %

MAR$_6$ = 2/30 = 6.67%

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	
1300-15-07-0~~0~~-1							✓						
2					✓		✓		✓	✓			
3	✓	✓		✓			✓	✓	✓	✓			
4													STA
5	✓			✓			✓						
6							✓						
7													STA

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR SAMPLING DATE AND TIME: 15-07-84
ANALYSIS DATE AND TIME: ANALYST:
SAMPLING LOCATION: [illegible]

③

Isolate Reference	AMP	C	CN	~~F~~ CAR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	KF	Remarks
1300-15-07-01-8	✓			✓									
9	✓	✓		✓		✓	✓		✓	✓			
10													STA
11	✓											✓	
12													STA
13	✓			✓		✓	✓	✓	✓	✓			
14													STA
15													STA
16													STA
17													STA
18							~~✓~~		✓	✓			
19							~~✓~~					✓	
20							✓						
21							~~✓~~						STA
22	✓			✓		✓	~~✓~~		✓	✓	✓		
23							~~✓~~						STA
24							~~✓~~		✓	✓			
25							~~✓~~						STA
26							✓			✓			
27													STA
28	✓			✓		✓	✓		✓	✓			
1300-15-07-01-29							~~✓~~						STA
30						✓	~~✓~~						
	n = 30		R = $\overset{17}{\cancel{21}}/30$ - 70.0 %				MAR_2 = 11/30 = 36.7%						
							MAR_3 = 7/30 = ~~20.0%~~ 23.3%						
							MAR_4 = 6/30 = 20.0%						
							MAR_5 = 5/30 = 16.7%						
							MAR_6 = 5/30 = 16.7%						
1300-15-07-02-1	✓			✓			✓		✓	✓			
2													STA
3													STA
4													STA
5	✓			✓		✓							
6													STA
7													STA
Dead ~~8~~													~~STA~~
9													STA
10							✓						
11													STA
12	✓			✓		✓	✓		✓	✓			
13	✓			✓					✓	✓			
14	✓			✓									~~STA~~

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: [illegible] .... ⑤

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~PT~~ F	S	SF	SXT	TE	~~POT~~ OT	K	Remarks KF	
1300-15-07-02-15													STA
16													STA
17													STA
18						✓			✓	✓			
19													STA
20													STA
21													STA
22							✓						
23	✓			✓		✓			✓				
24													STA
25													STA
odd morphology ~~26~~													
27													STA
28													STA
29						✓	✓		✓	✓	✓		
30													STA
	n=28	R= 10/28 = 35.7%					MAR$_2$ = 8/28 = 28.6% MAR$_3$ = 7/28 = 25.0% MAR$_4$ = 5/28 = 17.9% MAR$_5$ = 3/28 = 10.7%						
1300-15-07-0.51						✓			✓	✓	✓		
2													STA
3													STA
4						✓			✓	✓	✓		
5													STA
6													STA
7						✓			✓	✓			
8							✓		✓	✓			
9							✓					✓	
10													STA
11							✓		✓	✓			
12									✓	✓			
13						✓			✓	✓			
14						✓	✓		✓	✓	✓		
15													STA
16						✓	✓		✓	✓	✓		
17						✓	✓		✓	✓	✓		
18						✓			✓	✓	✓		
19													STA
20	✓			✓		✓							
21													STA

ANTIBIOTIC-RESISTANCE RECORD

⑨

PROJECT: GOLDBAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: ....

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks KF	
1300-15-07-0.5-22													STA
23													STA
24													STA
25	✓			✓		✓	✓		✓	✓			
26								✓		✓			
27						✓	✓		✓	✓	✓		
28						✓							
29													STA
30								✓	✓	✓			
	n=	30	R- 18/30 = 60.0%			MAR$_2$ = 17/30 = 56.7%							
						MAR$_3$ = 14/30 = 46.7%							
						MAR$_4$ = 8/30 = 26.7%							
						MAR$_5$ = 5/30 = 16.7%							
						MAR$_6$ = 1/30 = 3.33%							
1300-15-07-6.0-1													STA
2											✓		
3							✓						
4						✓	✓		✓	✓	✓		
5	✓	✓		✓		✓	✓		✓	✓	✓		
6													STA
7													STA
1300-15-07-1.0-8						✓	✓	✓	✓	✓	✓		
9							✓		✓	✓			
10					✓		✓		✓	✓			
dead ~~11~~													
12	✓			✓					✓	✓		✓	
13													STA
14	✓			✓		✓	✓		✓	✓	✓		
dead ~~15~~													
16						✓	✓		✓	✓	✓	✓	
17						✓	✓					✓	
18												✓	
19													STA
20					✓								
21													STA
22													STA
23													STA
24	✓			✓		✓			✓	✓			
25													STA
26													STA
27	✓			✓									
28						✓	✓				✓		

ANTIBIOTIC-RESISTANCE RECORD

PROJECT: GOLDBAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE AND TIME: .... ANALYST: ....
SAMPLING LOCATION: .... ⑨

Isolate Reference	AMP	C	CN	~~P~~ CAR	~~PT~~ F	S	SF	SXT	TE	~~TOB~~ OT	K	Remarks KF	
1300-15-07-10-29	✓			✓		✓					✓		
30	✓			✓					✓	✓			
	n = 28		R = 20/28 = 71.4%			MAR_2 = 14/28 = 50.0%							
						MAR_3 13/28 = 46.4%							
						MAR_4 10/28 = 35.7%							
						MAR_5 7/28 = 25.0%							
						MAR_6 4/28 = 14.3%							
1300-15-07-2.3-1													STA
2						✓	✓		✓	✓			
3													STA
4						✓			✓	✓			
5						✓			✓	✓			
6													STA
7						✓							
8													STA
9													STA
10													STA
11													STA
~~12~~ dead													
13												✓	
14													STA
~~1300-15-07-2.3-15~~ dead													
16	✓			✓		✓	✓						
17													STA
18													STA
19	✓			✓		✓			✓	✓		✓	
20													STA
21													STA
	n = 19		R = 7/19 = 36.8%			MAR_2 = 5/19 = 26.3%						Summary 1300-15-07-2.3	
MAR_3 = 5/19 = 26.3%			MAR_4 = 3/19 = 15.8%			MAR_5 = 1/19 = 5.26%						MAR_6 = 1/19	= 5.26%
1300-15-07-5.0-1													STA
2						✓	✓						
3													STA
4													STA
5	✓			✓		✓			✓	✓			
6													STA
7						✓	✓						
8													STA
9													STA
10									✓	✓			
	n = 10		R = 4/10 = 40.0%			MAR_2 = 4/10 = 40%				MAR_3 = 1/10 = 10%			

MAR_4 = 1/10 = 10% MAR_5 = 1/10 = 10% MAR_6 = 0/10 = 0%

Summary
1300-15-07-5.

ANTIBIOTI(ESISTANCE RECORD

PROJECT: GOLDRAR .... SAMPLING DATE AND TIME: 11-08-86 @ 11.00
ANALYSIS DATE: 22-08-86 .... ANALYST: BXU
SAMPLING LOCATION: GROUNTAIN ....

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	REMARKS
1100-11-08-0.0-1													STA
2				✓									
3	✓			✓		✓	✓		✓	✓			
4													STA
5	✓			✓									
6							✓	✓	✓	✓	✓		
7	✓			✓									
8	✓												
9													STA
10						/							STA
11													STA
12													STA
13	✓												
14													STA
15													STA
16	✓							✓		✓			
17													STA
18	Dead plate												
19													STA
20	✓	✓		✓	✓	✓			✓	✓	✓		
21													STA
1100-11-08-0.0 22													STA
23													STA
24											✓		
25													STA
26													STA
27	✓			✓		✓	✓		✓	✓			
28									✓	✓			
29	✓					✓			✓	✓			
30	✓			✓		✓	✓		✓	✓			

n = 29 R = 15/29 = 51.7% MAR_2 = 10/29 = ~~33.3%~~ 34.5% Summary
MAR_3 = 7/29 = ~~23.3%~~ 24.1% 1100-11-08-0.0
MAR_4 = 6/29 = ~~20.0%~~ 20.7%
MAR_5 = 5/29 = ~~16.7%~~ 17.0%
MAR_6 = 4/29 = 13.8%

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	REMARKS
1100-11-08-05-1													STA
2													STA
3						✓							
4	✓			✓									
5					✓	✓	✓		✓	✓	✓		
6	✓			✓		✓	✓						
7													STA

ANTIBIOTI ESISTANCE RECORD

PROJECT: GOLD.BAR SAMPLING DATE AND TIME:
ANALYSIS DATE: ANALYST:
SAMPLING LOCATION: ③

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	REMARKS
1100-11-08-05-8													STA
9													STA
10													STA
11													STA
12													STA
13						✓	✓						
14													STA
15													STA
16	✓			✓									
17													STA
18													STA
19													STA
20													STA
21					✓				✓	✓			
22													STA
23													STA
24													STA
25	✓			✓			✓	✓					
26													STA
27													STA
28													STA
1100-11-08-05-29	✓			✓								✓	
30													STA
	n = 30		R = 9/30 = 30.0%		MAR$_2$ = 8/30 = 26.7%								Summary
					MAR$_3$ = 4/30 = 13.3%								1100-11-08-05
					MAR$_4$ = 3/30 = 10.0%								
					MAR$_5$ = 1/30 = 3.33%								
					MAR$_6$ = 1/30 = 3.33%								
1100-11-08-10-1						✓	✓		✓	✓	✓		
2													STA
~~3~~	~~Dead plate~~												~~STA~~
4							✓						
5						✓	✓						~~STA~~
6													STA
7							~~✓~~						STA
8							✓						~~STA~~
9									~~✓~~				STA
10							✓		✓	✓			
11													STA
12							✓						
13													STA
14	✓			✓									

ANTIBIOTIC RESISTANCE RECORD

PROJECT: GOLD BAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE: .... ANALYST: ....
SAMPLING LOCATION: .... ⑤

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	REMARKS
1100-11-08-10-15													STA
16							✓						
17													STA
18													STA
19													STA
20							✓		✓	✓			
21													STA
22	✓			✓		✓	✓		✓	✓	✓		
23													STA
24	✓		✓	✓	✓	✓						✓	
25													STA
26													STA
27													STA
28													STA
29													STA
30													STA
	n = 29		R = 11/29 = 37.9 %				MAR$_2$ = 7/29		= 24.1%				Summary
							MAR$_3$ = 5/29		= 17.0%				1100-11-08-1.0
							MAR$_4$ = 3/29		= 10.3%				
							MAR$_5$ = 3/29		= 10.3%				
1100-11-08-20-1													STA
2									✓	✓			
3													STA
4	✓								✓	✓			
5													STA
6													STA
7													STA
8						✓	✓						
9													STA
10						✓			✓	✓			
11													STA
12													STA
13						✓	✓		✓	✓	✓		
14													STA
15													STA
16													STA
17	✓			✓								✓	
18													STA
19													STA
20													STA
21													STA

7

ANTIBIOTI ESISTANCE RECORD

PROJECT: GOLDBAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE: .... ANALYST: ....
SAMPLING LOCATION: .... ⑦

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	REMARKS
1100-11-08-20-22						✓	✓		✓	✓			
23	✓			✓							✓		
24													STA
25													STA
26									✓	✓			
27						✓			✓	✓			
28													STA
29													STA
30													STA
	n = 30		R = 10/30 = 33.3 %				MAR$_2$ = 10/30 = 33.3%						
							MAR$_3$ = 7/30 = 233%						
							MAR$_4$ = 2/30 = 667%						
							MAR$_5$ = 1/30 = 3.33%						
							MAR$_6$ = 0/30 = 0 %						
1100-11-08-5.0-1													STA
2					✓								
3													STA
4													STA
5													STA
6							✓		✓	✓			
7													STA
1100-11-08-5.0-8	✓			✓		✓			✓	✓	✓		
9	✓	✓		✓		✓	✓		✓	✓	✓		
10													STA
11													STA
12													STA
13													STA
14						✓	✓		✓	✓			
15	✓				✓	✓	✓		✓	✓		✓	
16	✓	✓		✓		✓	✓	✓	✓	✓	✓		
17	✓			✓		✓	✓		✓	✓			
18													STA
19	✓			✓								✓	
20	✓			✓								✓	
21													STA
22													STA
23													STA
24					✓								
25													STA
26						✓							
27													STA
28													STA

ANTIBIOTIC RESISTANCE RECORD

PROJECT: GOLDBAR.............. SAMPLING DATE AND TIME:....................
ANALYSIS DATE:................................. ANALYST:..........................
SAMPLING LOCATION:.. ⑨

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	REMARKS
1100-11-08-50-29									✓	✓			
30													STA
	n = 30			R = 13/30 = 43.3%			MAR$_2$ = 10/30 = 33.3%						
							MAR$_3$ = 9/30 = 30.0%						
							MAR$_4$ = 6/30 = 20.0%						
							MAR$_5$ = 5/30 = 16.7%						
							MAR$_6$ = 5/30 = 16.7%						
1100-11-08-100-1													STA
2													STA
3													STA
4													STA
5													STA
6													STA
7													STA
8													STA
9							✓						
10									✓	✓			
11													STA
12													STA
13													STA
14							✓						
1100-11-08-100-15													STA
16							✓		✓	✓			
17													STA
18													STA
19													STA
20							✓						~~STA~~
21													STA
22													STA
23													STA
24													STA
25													STA
26						✓			✓	✓			
27		✓											
28	✓			✓		✓	✓		✓	✓	✓		
29													STA
30												✓	
31													STA
32	✓			✓			✓						
33									✓	✓			
34									✓	✓			
35						✓							

ANTIBIOTI ESISTANCE RECORD

PROJECT: GOLDBAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE: .... ANALYST: ....
SAMPLING LOCATION: ....

⑪

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	REMARKS
1100-11-08-10036													STA
37													STA
38	✓	✓		✓		✓	✓						
39													STA
40													STA
	n = 40	R = 14/40 = 35.0 %					MAR$_2$ = 8/40 = ~~2.00%~~ 20.0%						
							MAR$_3$ = 5/40 = 12.5%						
							MAR$_4$ = 2/40 = 5.00%						
							MAR$_5$ = 2/40 = 5.00%						
							MAR$_6$ = 1/40 = 2.50%						
12-11-08-0.01													STA
2													STA
3													STA
4													STA
5							✓						
6													STA
7													STA
8	✓	✓		✓		✓	✓		✓	✓			
9									✓	✓			
10													STA
11							✓						
12													STA
13	✓			✓		✓	✓		✓	✓			
14							✓						
15													STA
16	✓			✓			✓						
17						✓	✓		✓	✓			
18							✓		✓	✓			
19	✓			✓	✓	✓							
20													STA
21													STA

ANTIBIOTIC RESISTANCE RECORD

PROJECT: GOLD BAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE : .... ANALYST: ....
SAMPLING LOCATION: .... ②

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	REMARKS
12-11-08-0.0-22													STA
23													STA
24													STA
25													STA
26							✓						STA
27													STA
28													STA
29													
30	✓			✓		✓			✓	✓			

n= 30 R= 13/30 = 43.3% MAR$_2$ = 8/30 = 26.7% Summary
MAR$_3$ = 7/30 = 23.3% 1200-11-08-0.0
MAR$_4$ = 5/30 = 16.7%
MAR$_5$ = 3/30 = 10.0%
MAR$_6$ = 2/30 = 6.67%

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	REMARKS
12-11-08-05-1	✓			✓			✓					✓	
2													STA
3							✓	✓	✓	✓			
4													STA
5	✓			✓		✓							
6													STA
12-11-08-0.5-7							✓		✓	✓			
8													STA
9							✓		✓	✓			
10													STA
11						✓			✓	✓			
12	✓			✓		✓			✓	✓			
13													STA
14						✓					✓		
15									✓	✓			
16													STA
17													STA
18													STA
19													STA
20	✓	✓		✓			✓		✓	✓			
21													STA
22													STA
23							✓		✓	✓			
24													STA
25													STA
26													STA
27	✓	✓		✓		✓	✓		✓	✓	✓	✓	

ANTIBIOTIC RESISTANCE RECORD

PROJECT: GOLDBAR .... SAMPLING DATE AND TIME: R ....
ANALYSIS DATE: .... ANALYST: ....
SAMPLING LOCATION: .... ④

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	REMARKS
12-11-08-05-28	✓			✓					✓	✓			
29													STA
30						✓							
	n = 30	R = 14/30 = 46.7 %				MAR_2 = 13/30 = 43.3 %							Summary
						MAR_3 = 11/30 = 36.7 %							1200-11-08-05
						MAR_4 = 6/30 = 20.0 %							
						MAR_5 = 3/30 = 10.0 %							
						MAR_6 = 2/30 = 6.67 %							
						~~MAR_7~~							
						~~MAR_8~~							
12-11-08-10-1							✓						
2													STA
3													STA
4													STA
5	✓			✓									
6							✓						
7													STA
8													STA
9	✓			✓									
10													STA
11									✓	✓			
12-11-08-10-12													STA
13						✓			✓	✓			
14						✓			✓	✓			
15									✓	✓			
16									✓	✓			
17	✓			✓		✓	✓		✓	✓	✓	✓	
18													STA
19													STA
20													STA
21													STA
22	✓			✓		✓	✓		✓	✓			
23													STA
24													STA
25													STA
26													STA
27													STA
28													STA
29													STA
30						✓	✓		✓	✓			
	n = 30	R = 12/30 = 40.0 %				MAR_2 = 10/30 = 33.3 %			MAR_3 = 5/30 = 16.7 %				Summary 1200-11-08

MAR_4 = 3/30 = 10.0 % MAR_5 = 2/30 = 6.67 % MAR_6 = 2/30 = 6.67 % 1.0

ANTIBIOTIC RESISTANCE RECORD

PROJECT: GOLD BAR .... SAMPLING DATE AND TIME: ....
ANALYSIS DATE: .... ANALYST: ....
SAMPLING LOCATION: .... ⑥

Isolate Reference	AMP	C	CM	CAR	F	S	SF	SXT	TE	OT	K	KF	REMARKS
12-11-08-20-1						✓			✓	✓			
2													STA
3	✓											✓	
4													STA
5													STA
6	✓			✓									
7													STA
8													STA
9										✓			
10													STA
11							✓		✓	✓			
12									✓	✓			
13	✓			✓									
14	✓			✓			✓			✓			
15													STA
16													STA
17													STA
18	✓			✓		✓	✓		✓	✓			
19													STA
20							✓		✓	✓			
21													STA
12-11-08-20-22	✓	✓		✓		✓	✓	✓	✓	✓			
23	✓			✓		✓			✓	✓			
24									✓	✓			
25													STA
26													STA
27	✓			✓									
28													STA
29													STA
30													STA
	n=30	R = 14/30 = 46.7 %				MAR_2 = 13/30 = 43.3%							Summary
						MAR_3 = 7/30 = 23.3%							1200-11-08-20
						MAR_4 = 4/30 = 13.3%							
						MAR_5 = 3/30 = 10.0%							
						MAR_6 = 2/30 = 6.67%							
12-11-08-50-1													STA
2	✓			✓		✓	✓					✓	
3													STA
4													STA
5													STA
6						✓			✓	✓			
7													STA

ANTIBIOTIC RESISTANCE RECORD

PROJECT: GOLD BAR SAMPLING DATE AND TIME:
ANALYSIS DATE: ANALYST:
SAMPLING LOCATION: ⑧

Isolate Reference	AMP	C	CN	CAR	F	S	SF	SXT	TE	OT	K	KF	REMARKS
12-11-08-50-8													STA
9													STA
10													STA
11							✓						
12													STA
13													STA
14													STA
15													STA
16	✓			✓									
17													STA
18									✓	✓			
19													STA
20						✓			✓	✓			
21									✓	✓			
22													STA
23							✓						
24													STA
25													STA
26													STA
27													STA
28	✓	✓		✓			✓		✓	✓			
12-11-08-50-29													STA
30													STA
	n= 30	R- 9/30 -30.0%				MAR_2 = 7/30 =			23.3%				Summary
						MAR_3 = 4/30 =			13.3%				12-11-08-5.0
						MAR_4 - 2/30 -			6.67%				
						MAR_5 = 2/30 =			6.67%				
						MAR_6 = 1/30 =			3.33%				
12-11-08-10.0-1	✓			✓		✓	✓		✓	✓			
2		✓					✓		✓	✓	✓		
3							✓						
4						✓			✓	✓			
5													STA
6						✓	✓					✓	
7					✓				✓	✓			
8													STA
9						✓			✓	✓			
10	✓	–		✓		✓					✓		
11							✓		✓	✓			
12							✓		✓	✓			
13													STA
14									✓	✓			

ANTIBIOTI(ESISTANCE RECORD

PROJECT: GOLDBAR SAMPLING DATE AND TIME:
ANALYSIS DATE: ANALYST:
SAMPLING LOCATION: ⑩

Isolate Reference	AMP	C	CN	CAR	F	S	SP	SXT	TE	OT	K	KF	REMARKS
12-11-08-100-15													STA
16							✓		✓	✓			
17													STA
18							✓						
19													STA
20						✓							
21	✓			✓			✓						
22													STA
23													STA
24						✓			✓	✓			
25							✓						
26									✓	✓			
27									✓	✓			
28													STA
29	✓			✓			✓	✓					
30					~~✓~~		~~✓~~	~~✓~~	~~✓~~	~~✓~~			STA
31					✓		✓	✓	✓	✓	[illegible]		
32									✓	✓	✓		[illegible]
33	~~✓~~		~~✓~~	~~✓~~	~~✓~~	~~✓~~			~~✓~~	~~✓~~		~~✓~~	STA
34	✓		✓	✓	✓	✓	[illegible]		✓	✓		✓	
35						✓	✓		✓	✓			
12-11-08-100-36													STA
37													STA
38													STA
39	✓			✓		✓							
40													STA

n= 40

R = 25/40 = 62.5%

MAR$_2$ = 21/40 = 52.5%

MAR$_3$ = 18/40 = 45.0%

MAR$_4$ = 7/40 = 17.5%

MAR$_5$ - 4/40 = 10.0%

MAR$_6$ = 2/40 = 5.00%

Summary
12-11-08-10.0

APPENDIX F: DISINFECTION DATA FROM THE DOSE-RESPONSE TRIALS IN OZONE DEMAND-FREE PHOSPHATE BUFFER

1. RUN NO.	2. D LEVEL	3. A DOSE mg/L	4. EC No./dL	5. EC 0 No./dL	11. T LEVEL	12. TIME s
23	1	.81000	11.000	.22000 +8	2	60.000
24	1	.81000	2.8000	.17000 +8	2	60.000
27	1	.87000	7.3000	.90000 +7	2	60.000
28	1	.87000	16.000	.17000 +8	2	60.000
77	1	.80000	21.000	.15000 +8	1	30.000
78	1	.80000	6.1000	.16000 +8	3	120.00
79	1	.78000	6.3000	.18000 +8	3	120.00
80	1	.78000	13.000	.16000 +8	1	30.000
81	1	.79000	4.5000	.14000 +8	3	120.00
82	1	.79000	6.0000	.17000 +8	1	30.000
2	2	.20000	62.000	.12000 +8	2	60.000
3	2	.22000	34.000	.15000 +8	2	60.000
4	2	.19000	68.000	.10000 +8	2	60.000
5	2	.18000	170.00	.15000 +8	2	60.000
7	2	.20000	150.00	.13000 +8	2	60.000
47	2	.20000	52.000	.17000 +8	2	60.000
50	2	.21000	46.000	.18000 +8	2	60.000
54	2	.20000	430.00	.18000 +8	1	30.000
57	2	.20000	10.000	.17000 +8	3	120.00
63	2	.19000	1300.0	.19000 +8	1	30.000
65	2	.20000	21.000	.15000 +8	3	120.00
73	2	.20000	17.000	.21000 +8	3	120.00
9	4	.48000 -1	3200.0	.10000 +8	2	60.000
11	4	.51000 -1	770.00	.16000 +8	2	60.000
13	4	.46000 -1	830.00	.18000 +8	2	60.000
45	4	.42000 -1	11000.	.13000 +8	2	60.000
55	4	.46000 -1	6500.0	.19000 +8	1	30.000
60	4	.42000 -1	45.000	.17000 +8	3	120.00
62	4	.43000 -1	5200.0	.21000 +8	1	30.000
67	4	.44000 -1	240.00	.21000 +8	3	120.00
70	4	.46000 -1	5200.0	.21000 +8	1	30.000
75	4	.45000 -1	190.00	.20000 +8	3	120.00
19	6	.11000 -1	1900.0	.18000 +8	2	60.000

1. RUN NO.	2. D LEVEL	3. A DOSE mg/L	4. EC No./dL	5. EC O No./dL	11. T LEVEL	12. TIME s
34	6	.12000 -1	3500.0	.16000 +8	2	60.000
37	6	.12000 -1	2500.0	.18000 +8	2	60.000
46	6	.11000 -1	34000.	.16000 +8	2	60.000
48	6	.11000 -1	33000.	.18000 +8	2	60.000
52	6	.11000 -1	34000.	.19000 +8	2	60.000
56	6	.11000 -1	6000.0	.17000 +8	1	30.000
58	6	.10000 -1	4100.0	.17000 +8	3	120.00
61	6	.11000 -1	5800.0	.20000 +8	1	30.000
68	6	.11000 -1	12000.	.17000 +8	3	120.00
69	6	.11000 -1	5000.0	.21000 +8	1	30.000
76	6	.11000 -1	2800.0	.19000 +8	3	120.00
25	7	.43000 -2	8100.0	.16000 +8	2	60.000
26	7	.43000 -2	7300.0	.18000 +8	2	60.000
31	7	.43000 -2	14000.	.17000 +8	2	60.000
32	7	.43000 -2	14000.	.16000 +8	2	60.000
44	7	.43000 -2	22000.	.13000 +8	2	60.000
49	7	.45000 -2	33000.	.14000 +8	2	60.000
51	7	.45000 -2	3400.0	.19000 +8	2	60.000
53	7	.47000 -2	.54000 +7	.17000 +8	1	30.000
59	7	.43000 -2	7100.0	.17000 +8	3	120.00
64	7	.44000 -2	.69000 +7	.10000 +8	1	30.000
66	7	.43000 -2	4300.0	.18000 +8	3	120.00
72	7	.45000 -2	.24000 +7	.20000 +8	1	30.000
74	7	.45000 -2	11000.	.19000 +8	3	120.00

NOTE:

D LEVEL = Dose level

A DOSE = Applied ozone dose

T LEVEL = Time level

APPENDIX G: DISINFECTION DATA FROM THE DOSE-RESPONSE TRIALS IN SECONDARY EFFLUENT AT BENCH-SCALE

1. RUN NO.	2. TRIAL NO	3. D LEVEL *	6. T DOSE mg/L	7. EC No./dL	8. EC O No./dL	4. T LEVEL	5. TIME s
1	1	1	9.5000	880.00	.20000 +8	1	60.000
1	5	1	9.9000	500.00	.23000 +8	2	600.00
2	1	1	9.5000	480.00	.22000 +8	1	60.000
2	5	1	9.4000	420.00	.25000 +8	2	600.00
3	2	1	9.1000	630.00	.20000 +8	1	60.000
3	5	1	9.1000	-0.	.20000 +8	2	600.00
1	4	2	4.8000	1100.0	.19000 +8	2	600.00
1	7	2	5.2000	1200.0	.18000 +8	1	60.000
2	3	2	4.9000	680.00	.23000 +8	1	60.000
2	6	2	5.0000	680.00	.22000 +8	2	600.00
3	1	2	4.6000	620.00	.16000 +8	2	600.00
3	8	2	5.0000	660.00	.19000 +8	1	60.000
1	2	3	2.5000	.51000 +7	.22000 +8	2	600.00
1	6	3	2.4000	.67000 +7	.22000 +8	1	60.000
2	4	3	2.5000	.58000 +7	.18000 +8	1	60.000
2	8	3	2.4000	.68000 +7	.18000 +8	2	600.00
3	4	3	2.3000	.72000 +7	.17000 +8	1	60.000
3	7	3	2.4000	.55000 +7	.19000 +8	2	600.00
1	3	4	1.2000	.16000 +8	.25000 +8	1	60.000
1	8	4	1.3000	.13000 +8	.26000 +8	2	600.00
2	2	4	1.3000	.83000 +7	.22000 +8	2	600.00
2	7	4	1.3000	.13000 +8	.23000 +8	1	60.000
3	3	4	1.3000	.12000 +8	.16000 +8	2	600.00
3	6	4	1.3000	.13000 +8	.22000 +8	1	60.000

NOTE: D LEVEL = Dose level
T DOSE = Transferred ozone dose
T LEVEL = Time level

APPENDIX H: DISINFECTION DATA FROM THE DOSE-RESPONSE TRIALS IN SECONDARY EFFLUENT AT PILOT-SCALE

1. RUN NO.	2. WW BATCH	3. D LEVEL	4. A DOSE mg/L	5. T DOSE mg/L	6. R DOSE mg/L	7. EC No./dL	8. EC 0 No./dL	16. U DOSE mg/L
1	1	6	0.	0.	0.	.13000 +8	.13000 +8	0.
1	1	5	1.5000	1.5000	0.	.12000 +8	.13000 +8	1.5000
1	1	4	3.1000	3.1000	0.	.54000 +7	.13000 +8	3.1000
1	1	3	6.1000	6.1000	.30000	270.00	.13000 +8	5.8000
1	1	2	12.000	12.000	1.7000	41.000	.13000 +8	10.300
1	1	1	25.000	23.000	2.6000	2.5000	.13000 +8	22.400
2	2	6	0.	0.	0.	.12000 +8	.12000 +8	0.
2	2	5	1.5000	1.5000	0.	.12000 +8	.12000 +8	1.5000
2	2	4	3.0000	3.0000	0.	.27000 +7	.12000 +8	3.0000
2	2	3	6.0000	6.0000	.10000	-0.	.12000 +8	5.9000
2	2	2	12.000	12.000	.70000	67.000	.12000 +8	11.300
2	2	1	23.000	22.000	1.2000	12.000	.12000 +8	21.800
3	3	6	0.	0.	0.	.13000 +8	.13000 +8	0.
3	3	5	1.4000	1.4000	0.	.92000 +7	.13000 +8	1.4000
3	3	4	2.9000	2.9000	.10000	50000.	.13000 +8	2.8000
3	3	3	5.9000	5.9000	.30000	130.00	.13000 +8	5.6000
3	3	2	12.000	12.000	1.0000	21.000	.13000 +8	11.000
3	3	1	22.000	21.000	1.7000	2.5000	.13000 +8	20.300
4	3	6	0.	0.	0.	.12000 +8	.12000 +8	0
4	3	5	1.3000	1.3000	0.	.83000 +7	.12000 +8	1.3000
4	3	4	2.8000	2.8000	.10000	50000.	.12000 +8	2.7000
4	3	3	5.6000	5.6000	.30000	100.00	.12000 +8	5.3000
4	3	2	11.000	11.000	1.0000	18.000	.12000 +8	10.000

1. RUN NO.	2. WW BATCH	3. D LEVEL	4. A DOSE mg/L	5. T DOSE mg/L	6. R DOSE mg/L	7. EC No./dL	8. EC O No./dL	16. U DOSE mg/L
4	3	1	21.000	20.000	1.7000	4.0000	.12000 +8	19.300
5	4	6	0.	0.	0.	.12000 +8	.12000 +8	0.
5	4	5	1.4000	1.4000	0.	.10000 +8	.12000 +8	1.4000
5	4	4	2.8000	2.8000	0.	.92000 +6	.12000 +8	2.8000
5	4	3	5.7000	5.7000	.20000	740.00	.12000 +8	5.5000
5	4	2	11.000	11.000	.90000	220.00	.12000 +8	10.100
5	4	1	22.000	21.000	1.7000	10.000	.12000 +8	20.300
6	4	6	0.	0.	0.	.12000 +8	.12000 +8	0.
6	4	5	1.6000	1.6000	0.	.10000 +8	.12000 +8	1.6000
6	4	4	3.2000	3.2000	0.	.23000 +7	.12000 +8	3.2000
6	4	3	6.3000	6.3000	.20000	740.00	.12000 +8	6.1000
6	4	2	13.000	13.000	.80000	150.00	.12000 +8	12.200
6	4	1	24.000	23.000	1.6000	6.0000	.12000 +8	22.400
7	5	3	6.0000	6.0000	.20000	230.00	.15000 +8	5.8000
8	5	3	6.0000	6.0000	.20000	280.00	.12000 +8	5.8000
9	6	3	6.0000	6.0000	.20000	180.00	.14000 +8	5.8000
10	6	3	6.0000	6.0000	.20000	230.00	.15000 +8	5.8000

NOTE: A DOSE = Applied ozone T DOSE = Transferred ozone U DOSE = Utilized ozone
R DOSE = Residual ozone

APPENDIX I: DISINFECTION DATA FROM THE TRIALS DETERMINING THE EFFECT OF MIXING INTENSITY AND STORAGE CONDITIONS ON PERFORMANCE

5. WW BATCH	2. SAMPLE	3. STORAGE	4. SPEED	1. EC No./dL
1	1	1	1	270.00
1	2	1	1	190.00
1	3	1	1	-0.
1	4	1	1	-0.
1	5	1	1	-0.
1	1	2	1	19000.
1	2	2	1	19000.
1	3	2	1	16000.
1	4	2	1	19000.
1	5	2	1	17000.
1	1	1	2	310.00
1	2	1	2	360.00
1	3	1	2	180.00
1	4	1	2	210.00
1	5	1	2	400.00
1	1	2	2	18000.
1	2	2	2	13000.
1	3	2	2	17000.
1	4	2	2	18000.
1	5	2	2	15000.
2	1	1	1	160.00
2	2	1	1	140.00
2	3	1	1	200.00
2	4	1	1	200.00
2	5	1	1	190.00
2	1	2	1	14000.

5. WW BATCH	2. SAMPLE	3. STORAGE	4. SPEED	1. EC No./dL
2	2	2	1	12000.
2	3	2	1	12000.
2	4	2	1	13000.
2	5	2	1	13000.
2	1	1	2	260.00
2	2	1	2	270.00
2	3	1	2	190.00
2	4	1	2	250.00
2	5	1	2	190.00
2	1	2	2	9800.0
2	2	2	2	8200.0
2	3	2	2	10000.
2	4	2	2	8400.0
2	5	2	2	9800.0

NOTE: SAMPLE = Sample number

STORAGE = 1 - Quiescent condition
2 - Stirred condition

SPEED = 1 - 200 rpm
2 - 300 rpm

APPENDIX J: CALCULATIONS FOR DETERMINATION OF OZONE CONCENTRATION IN PROCESS GAS

Gas Phase

Ozone Concentration Relationships

STP = 273.15° K, 101.33 kPa

$$C = \frac{\text{mass of ozone}}{L_g} \quad @\ STP$$

$$\rho_{O3} = 2.144\ \frac{g}{L}\ @\ STP \qquad \rho_{O2} = 1.429\ \frac{g}{L}\ @\ STP$$

$$C = \frac{1429 * w/w\ \%}{100-0.333 * w/w\ \%} \quad @\ STP$$

$$\frac{V}{V}\ \% = 0.0466667\ C \quad @\ STP$$

$$\frac{V\%}{V} = 1.4921\ \frac{w\%}{w} \quad @\ STP$$

$$\text{ppm-V} = 1.0 \times 10^{-4}\ (v/v\%)$$

Calculating Ozone Concentration in the Gas Phase

by Iodometry: Calculation Sheet

1. Correct O_2 Volume to STP (273°K; 760 mm Hg)

Ta = ________ °K   Pa = ________ mm Hg Pw_{Ta} = ________ mm Hg

Correction Factor F

$$F = \frac{273°K}{Ta} * \frac{(Pa + Pwt) - Pw_{Ta}}{760 \text{ mm Hg}}$$

(Pwt) = wet test meter pressure)

$V_{STP} = Vm * F$ is O_2 volume at STP

(Vm = measured volume using wet test meter)

2. Mass of Ozone in Carrier Gas

M_{O3} = ________ mL titrant * ________ N titrant * 0.024 g

= ________ g

V_{O3} = ________ g * $\frac{22.4 \text{ L}}{48 \text{ g}}$

= ________ L_{STP}

3. Concentration of Ozone in Carrier Gas at STP

$$C_{O3,STP} = \frac{\_\_\_\_\_\_\_\_ \text{ g}}{\_\_\_\_\_\_\_\_ L_{O2,STP} + \_\_\_\_\_\_\_\_ L_{O3,STP}}$$

= ________ g/L_{STP}

APPENDIX K: FORMAL EVALUATION OF LACK-OF-FIT FOR SELECTED DOSE-RESPONSE MODELS

Model [6.2]

$$\log \frac{N}{N_0} = -1.61 - 1.61 \log C_A$$

Source	d.f.	S.S.	M.S.	F
Model	1	99.535	99.535	
Error	55	32.018	0.58215	
L.o.F.	13	25.9511	1.9962	13.8 *
P.E.	42	6.0669	0.1445	
Total	56	131.55		

* $F_{(13,42,0.01)} \cong 2.6$ ∴ conclude there is L.O.F.

Model [6.7]

$$\log \frac{N}{N_0} = -0.681 - 3.98 \log C_u$$

Source	d.f.	S.S.	M.S.	F
Model	1	5.5721	5.5721	
Error	7	0.83691	0.11956	
L.O.F.	1	0.27999	0.27999	3.01 *
P.E.	6	0.55692	0.09282	
Total	8	6.4090		

* $F_{(1,6,0.01)} = 13.7$ ∴ conclude there is no L.O.F.

Model [7.4]

$$-\log \frac{N}{N_0} = 0.743 - 5.91\, C_A$$

Source	d.f.	S.S.	M.S.	F
Model	1	82.371		
Error	21	14.954	0.71208	
L.O.F.	2	14.648	7.324	455 *
P.E.	19	0.30590	0.01610	
Total	22			

* $F_{(2,19,0.01)} \approx 5.9$ ∴ conclude there is L.O.F.

NOTES:

1. P.E. = pure error
2. L.O.F. = Lack-of-fit
3. d.f. = degrees of freedom
4. S.S. = sum of squares
5. M.S. = mean square

APPENDIX L: CURRICULUM VITÆ

CURRICULUM VITÆ

GORDON R. FINCH, M.Sc., P.Eng.

CURRENT ACTIVITIES: (July 1984 to present)

Position:
Ph.D Candidate
Department of Civil Engineering
University of Alberta
Edmonton, Alberta, T6G 2G7
(403) 432-3950

Interests:
Alternate disinfection methods, sanitary microbiology, public health, cold regions environmental engineering, scale-up and scale-down of treatment processes, water and sanitation in developing countries, and statistical methods in research and engineering.

Research:
Ozone treatment of water and wastewater with detailed investigation into ozone disinfection of antibiotic-resistant *Escherichia coli* and a nalidixic acid resistant marker strain *E. coli.*

EDUCATION:

Ph.D., Environmental Engineering,
University of Alberta (expected fall 1987)

M.Sc., Environmental Engineering,
University of Alberta, 1984

B.A.Sc., Civil Engineering,
University of Toronto, 1976

PROFESSIONAL AFFILIATIONS:

Member, American Water Works Association
Member, Association of Professional Engineers, Geologists and Geophysicists of Alberta
Member, Canadian Society for Civil Engineering
Member, Engineering Institute of Canada
Member, International Association on Water Pollution Research and Control
Member, International Ozone Association
Member, Water Pollution Control Federation
Member, Western Canada Water and Sewage Conference

AWARDS:

Graduate Studies Fellowship, University of Alberta
Natural Sciences and Engineering Research Council Postgraduate Scholarship
Andrew Stewart ~~Graduate~~ Prize, University of Alberta
Natural Sciences and Engineering Research Council Postdoctoral Fellowship
Province of Alberta Graduate Fellowship

PROFESSIONAL ACTIVITIES:

1984 Oct-date:

Consulting to Daniel W. Smith and Associates on various environmental engineering projects. These include preparation of lagoon design, operation, and maintenance guidelines, preparation of the Cold Climate Utilities Manual, a feasibility study for the renovation of a northern lagoon, review and assessment of water filtration technology, evaluation of an on-site wastewater treatment system, and a microbiological water quality review of a water supply. Currently acting as technical advisor to the City of Edmonton's Water and Sanitation Department's water treatment process improvement team.

1986 Sept-Dec:

Sessional lecturer in the Department of Civil Engineering, University of Alberta. Prepared and presented a graduate course in the design and analysis of civil engineering experiments.

1983 Nov-1984 June:

Investigation into upgrading seasonal discharge sewage lagoons using batch chemical coagulation. The study focussed principally on the factors affecting the coagulation and flocculation processes with the goal of optimizing fecal coliform removal using alum.

1982 Dec-1983 Oct:

Research assistant with the Environmental Engineering group involved in preparing a critical review of cold climate sewage lagoon theory, performance, design, operation and maintenance for Environment Canada.

1980-82:

Project Engineer with Proctor, Redfern, Butler, and Krebes Consultants Limited, Edmonton, Alberta. Responsible for pre-engineering, detailed design of municipal services, estimation, contract administration, and construction supervision of residential land development projects in Edmonton and High Level, Alberta.

1977-79:

Senior Training Officer in the Department of Works and Supply for the Government of Papua New Guinea. The position involved developing, implementing, and operating a training program for field personnel in road construction, road maintenance, and heavy equipment operation. Responsibilities included establishment and management of a national training centre oriented towards practical, transfer training supported by classroom theory. Duties included managing a staff of eight people, acquistion of heavy equipment, control of a million dollar budget, preparing course syllabi, and ensuring that training projects were run effectively.

1976-77:

Graduate Engineer with The Proctor and Redfern Group, Toronto, Ontario. Assisted the resident engineer in construction supervision of major storm and sanitary trunk drainage systems for the new community of Erin Mills, Ontario. As a design engineer designed complete municipal services for residential land development projects.

REFEREED PUBLICATIONS:

Finch, G.R., M.E. Stiles, and D.W. Smith. 1987. Recovery of a marker strain of *Escherichia coli* from ozonated water by membrane filtration. *Applied and Environmental Microbiology* (accepted for publication).

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Heinke, G.W., D.W. Smith, and G.R. Finch. 1987. *Guidelines for the Planning, Design, Operation, and Maintenance of Sewage Lagoons in the Northwest Territories*. Prepared for the Government of the NWT.

Smith, D.W. (editor). 1986. *Cold Climate Utilities Manual*. Environment Canada and Government of the Northwest Territories. Contributor to various components of the manual.

Daniel W. Smith and Associates. 1986. *Task 2.2: Microbiological Water Quality of the North Saskatchewan River*. A proprietary report for Steve E. Hrudey and Associates, Edmonton, Alberta.

Heinke, G.W., D.W. Smith, and G.R. Finch. 1986. *Niven Lake Rehabilitation Study*. A report prepared for the City of Yellowknife, Northwest Territories.

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